Canadian Journal of Fisheries and Aquatic Sciences

(Formerly the *Journal of the Fisheries Research Board of Canada*)

Journal canadien des sciences halieutiques et aquatiques

(Anciennement le *Journal de l'office des recherches sur les pêcheries du Canada*)

Volume 37, No. 9, September 1980

Volume 37, nº 9, septembre 1980

Government of Canada
Fisheries and Oceans

Gouvernement du Canada
Pêches et Océans

Canadian
Journal
of Fisheries
and Aquatic
Sciences

Journal
canadien
des sciences
halieutiques
et aquatiques

Government of Canada
Fisheries and Oceans

Scientific Information
and Publications Branch
Ottawa K1A 0E6

Gouvernement du Canada
Pêches et Océans

Direction de l'information
et des publications scientifiques
Ottawa K1A 0E6

Volume 37, No. 9, September 1980

Volume 37, nº 9, septembre 1980

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial policy — The *Journal* publishes original research articles and notes, critical reviews, essays of opinion or hypothesis, letters, and book reviews in the broad field of fisheries management and technology, ocean science, and aquatic environments relevant to Canada. Submissions in English or French are accepted without limitations as to origin, but must not have been published or copyrighted and must not be published elsewhere except in abstract form or by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company. Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

A charge of $44 per page is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Subscriptions — The *Journal* is issued monthly to form annual volumes of twelve numbers. Subscription rates are: Canada, $47 per year; other countries, $56.40. Single issues are $4.00 and $4.80, respectively. Orders must be sent directly to Supply and Services Canada, Hull, Que., Canada, K1A 0S9, and payment made in Canadian funds to "The Receiver General for Canada."

Microfilm — Issues from 1934 through the current volume can be purchased on 16 or 35 mm microfilm. Photocopies of individual articles or issues can be purchased. Address orders to Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Publié depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres: *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–79.

Politique de publication — Le *Journal* publie des articles de recherches, ainsi que des notes, critiques, exposés d'opinions ou d'hypothèses, lettres et recensions concernant le vaste domaine de la gestion des pêches et de la technologie, des sciences de la mer et des milieux aquatiques au Canada. Toutes contributions en français ou en anglais sont acceptées sans restriction quant à la provenance, mais elles ne doivent pas avoir été produites ni avoir fait l'objet d'un copyright et ne doivent pas être publiées nulle part ailleurs sauf sous forme abrégée ou avec le consentement écrit du directeur. Le *Journal* décline toute responsabilité pour ce qui est des affirmations des collaborateurs. L'utilisation de noms de marque de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique. Les guides des auteurs et le guide des dactylos paraissent dans le premier numéro de chaque volume et sont disponibles gratuitement du *Journal*.

Les frais de publication sont de $44 la page. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés.

Abonnement — Le *Journal* paraît tous les mois, formant ainsi un volume de 12 numéros par année. Les frais d'abonnement annuel sont de $47 pour le Canada et de $56.40 pour les autres pays. Chaque numéro coûte $4.00 au Canada et $4.80 à l'étranger. Prière d'adresser directement les commandes à Approvisionnements et Services Canada, Hull (Qué.), Canada K1A 0S9; les paiements doivent être faits en devises canadiennes, à l'ordre du Receveur général du Canada.

Microfilms — On peut acheter sur microfilms 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente. Prière d'adresser les commandes à Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, E.-U.

Editor and Director of Scientific Information/ Rédacteur et Directeur de l'information scientifique	J. WATSON, PH.D.
Acting Deputy Editor/ Sous-rédactrice intérimaire	JOHANNA M. REINHART, M.SC.
Assistant Editors/ Rédacteurs adjoints	D. G. COOK, PH.D./ LORRAINE C. SMITH, PH.D.
Production-Documentation	J. CAMP/ G. J. NEVILLE/ PATRICIA A. LALLA/ A. T. REID
French Abstracts/ Résumés français	Y. JEAN, PH.D.
Book Review Editor/ Rédacteur des comptes rendus	D. P. SCOTT, PH.D.

Canadian Journal of Fisheries and Aquatic Sciences
Volume 37, No. 9, September 1980

Journal canadien des sciences halieutiques et aquatiques
Volume 37, n° 9, septembre 1980

A New Approach to Length–Frequency Analysis: Growth Structure

JON SCHNUTE AND DAVID FOURNIER

Department of Fisheries and Oceans, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

SCHNUTE, J., AND D. FOURNIER. 1980. A new approach to length–frequency analysis: growth structure. Can. J. Fish. Aquat. Sci. 37: 1337–1351.

This paper presents a new approach to length–frequency analysis which takes account of biological structure in the mean lengths and standard deviations in length for various age-classes of fish. The new methods help determine biologically meaningful solutions, even when earlier methods lead to an ambiguous set of competing solutions. The structure of the standard deviations turns out to be especially important. For describing the means, new parameters are defined for von Bertalanffy growth which prove to have greater biological meaning and numerical stability than L_∞, K, and t_0. These new parameters can often be estimated easily from the raw data in cases where the species experiences a slowing of growth with age. This paper also presents χ^2 methods which can be used to rank competing solutions, although the results are not definitive. All methods are illustrated using data previously published for pike and abalone. An appendix describes in detail the computer programs required for the analysis.

Key words: length–frequency analysis, aging of samples, von Bertalanffy growth, growth, maximum likelihood estimation, nonlinear estimation

SCHNUTE, J., AND D. FOURNIER. 1980. A new approach to length–frequency analysis: growth structure. Can. J. Fish. Aquat. Sci. 37: 1337–1351.

L'approche nouvelle de l'analyse de la fréquence des longueurs présentée dans cet article tient compte de la structure biologique dans les longueurs moyennes et dans les écarts types de longueur chez diverses classes d'âge de poissons. Ces nouvelles méthodes aident à trouver des solutions biologiquement significatives, même quand d'autres méthodes ont donné un ensemble ambigu de solutions opposées. Particulièrement importante est la structure des écarts types. De nouveaux paramètres de croissance de von Bertalanffy sont utilisés pour décrire les moyennes et s'avèrent biologiquement plus significatifs et numériquement plus stabes que L_∞, K et t_0. Souvent, ces nouveaux paramètres sont facilement estimés à partir des données brutes dans les cas où l'espèce subit un ralentissement de croissance avec l'âge. On trouvera également dans cet article un exposé de méthodes de χ^2 pouvant servir à classer des solutions opposées, sans toutefois qu'on ait de résultats définitifs. Toutes ces méthodes sont illustrées par des données déjà publiées sur le brochet et l'ormeau. Les programmes d'ordinateur nécessaires à l'analyses sont décrits en détail en appendice.

Received September 10, 1979
Accepted May 22, 1980

Reçu le 10 septembre 1979
Accepté le 22 mai 1980

Printed in Canada (J5772)
Imprimé au Canada (J5772)

In studying a fish stock, the biologist frequently tries to determine three basic characteristics, namely, (1) the mean length at each age, (2) the distribution of lengths, and (3) the distribution of ages. Clearly these characteristics are related. For example, if the mean length at age and the length distribution are both known, then something can be said about the age distribution. This principle underlies the use of age–length keys.

Usually the distribution of fish lengths is the easiest characteristic of the three to ascertain. One need only take a suitably large random sample of the stock and measure the length of each fish in it. By contrast, the other two characteristics may be very difficult to determine, because they involve knowing the ages of fish in a sample. For this reason attempts have been made to use the length distribution alone to determine both the age distribution and the mean length at age.

Such methods date back to Petersen (1892), who used length–frequency modes in a population of *Zoarces viviparus* to identify distinct age-groups. An interesting reproduction of Petersen's key figure appears in Ricker (1975, p. 204); one can see immediately how distinct length clusters visually suggest distinct ages. About the same time, Pearson (1894) developed the first statistical treatment of the problem of overlapping component distributions, although his method applied to the case of two components only and did not easily generalize. For practical reasons, modern computers are necessary to implement a general statistical procedure for distinguishing an arbitrary number of overlapping component distributions. Hasselblad (1966) first described such a method in detail and developed the necessary computer program. However, workers in fisheries research have primarily favored the graphical methods of Harding (1949) and Cassie (1954). In a recent departure from this tradition, Macdonald and Pitcher (1979) show how some variations of Hasselblad's statistical technique can be applied to fish stocks. Also, McNew and Sommerfelt (1978) discuss difficulties which may occur when the distribution of lengths at each age is not normal as Hasselblad assumes.

The principal motive for length–frequency analysis has usually been to determine the distribution of ages of fish. For management purposes, it is important to know the age composition to predict the status of the stock in future years. However, as stated earlier, the analysis also gives information on the length at age. The interested worker may then go on to construct growth curves, say of the von Bertalanffy type. This has always been viewed as a separate problem. First, one might use the method of Cassie to find mean lengths at age; then, second, one might determine an appropriate growth curve. These have been considered separate issues, with a distinct procedure for each case.

This paper presents a new point of view in which these two previously separate issues become one. A single procedure applied to length–frequency data gives both the age composition of the stock and the parameters for growth. More explicitly, the procedure uses length–frequency data to determine the percentage of fish at each age, as well as parameters which define the mean length and standard deviation in length at each age. For example, three von Bertalanffy parameters (such as L_∞, K, t_0) might be determined which define a growth curve through the mean lengths.

Aside from giving growth data along with the usual age composition analysis, the new procedure has at least one further advantage over those used previously. It helps eliminate ambiguity. Most workers who try Cassie's method of plotting the cumulative frequency on probability paper soon discover that the location of inflection points (which serve as breaking points between age-groups) can be quite ambiguous. Also, each time one makes an ambiguous choice, that choice perpetuates itself into the determination of further inflection points. This difficulty is somewhat circumvented by Hasselblad's statistical procedure, but not entirely. Even Hasselblad's method may give rise to many competing solutions, particularly when the number of age-groups is large and older fish with different ages have similar lengths. Macdonald and Pitcher deal with such problems by invoking ad hoc constraints on some of the parameters, especially the standard deviations. In this paper, we explore different constraints, motivated biologically. Instead of allowing arbitrary mean lengths and standard deviations in length as Hasselblad does, our procedure requires that the means and/or standard deviations should conform to a growth pattern. For many species this is reasonable because, for example, the growth rate slows as the fish mature and, consequently, the mean lengths are related to each other in some way.

A general conclusion from this argument is that more biological structure leads to less ambiguity. Unconstrained methods may give many possible solutions to the same problem. By requiring that population characteristics (such as mean lengths at age) must bear some relationship to one another on biological grounds, one can perhaps eliminate a significant number of possible solutions and focus immediately on a restricted number which are of biological interest. Although emphasis is placed on von Bertalanffy growth, other relationships among population characteristics (for example, between standard deviations and means) will also be introduced. In all cases, the underlying principle remains the same, namely, introducing biological structure into the statistical model to locate solutions of biological interest. In each application the research biologist may wish to tailor the model according to his own view of the biological characteristics of the stock.

One final point should be clarified before the analysis begins. The terms "age distribution" and "length distribution" apply throughout to a stock which has been randomly sampled. That population may be a stock at sea, but more often it is the total population of caught fish. If random samples are taken from the commercial catch and the gear is selective for certain ages or sizes of fish (as it almost always is), then, of course, the population at sea has not been randomly sampled. At best the results can speak for the hypothetical population of catchable fish, or, more simply, the entire catch itself. If inferences are to be made about a stock at sea, then the biologist may need to take samples with special noncommercial gear, or he may have to compensate his results from the commercial catch in some way to allow for gear selectivity.

von Bertalanffy Growth

Before introducing the structure of biological growth into the statistics of length–frequency, it is useful to take a fresh look at what is implied by von Bertalanffy growth. Suppose that the fish population being considered has M age-classes from age a_1 to a_M, where the ith age is

$$(1) \quad a_i = a_1 + i - 1; \qquad i = 1, \ldots, M.$$

Let μ_i be the mean length of fish at age a_i. Then, if these means lie on a curve of the type proposed by von Bertalanffy (1938),

$$(2) \quad \mu_i = L_\infty(1 - e^{-K(a_i - t_0)}); \qquad i = 1, \ldots, M.$$

In this equation, L_∞ is the theoretical length which fish approach as they grow older, K relates to the fraction by which the gap between current length and asymptotic length is reduced each year, and t_0 is the age at which fish length extrapolates back to zero along the curve.

As Ricker (1975) points out in connection with the work of Ford (1933) and Walford (1946), equation (2) implies that

$$(3) \quad \mu_{i+2} - \mu_{i+1} = e^{-K}(\mu_{i+1} - \mu_i).$$

Consequently, the distance between two successive mean lengths shrinks each year to the fraction

$$(4) \quad k = e^{-K}$$

of its former size. This reduction corresponds to a slowing in growth; as the fish get older, the change in mean length from one year to the next becomes smaller.

The idea behind equation (3) can be used to formulate a new version of the von Bertalanffy growth equation. Suppose that the first and final mean lengths μ_1 and μ_M are known. Call them l and L, respectively. Then from (1)–(2)

$$(5) \quad l = L_\infty(1 - e^{-K(a_1 - t_0)}),$$

$$(6) \quad L = L_\infty(1 - e^{-K(a_1 + M - 1 - t_0)}).$$

According to (3)–(4), the remaining means, from μ_2 to μ_{M-1}, must lie between l and L so that each year the incremental growth $\mu_{i+1} - \mu_i$ shrinks to the fraction k of its previous value. There is only one way to arrange the remaining means in this fashion. For example, if $k = 1$, the means must be equally spaced between l and L. If $k \neq 1$, it turns out that

$$(7) \quad \mu_i = l + (L - l)\frac{1 - k^{i-1}}{1 - k^{M-1}}; \qquad i = 1, \ldots, M.$$

This result is proved in Appendix A.

Equations (2) and (7) are merely two expressions for the same curve with different choices of parameters; (2) uses L_∞, K, t_0, while (7) uses l, L, k. Also, (4)–(6) gives the transformation from the first set of parameters to the second. The reverse transformation is given by

$$(8) \quad L_\infty = \frac{L - lk^{M-1}}{1 - k^{M-1}},$$

$$(9) \quad K = -\log k,$$

$$(10) \quad t_0 = a_1 - \frac{1}{\log k} \log\left(\frac{L - l}{L - lk^{M-1}}\right).$$

The parameter set (l, L, k) is much more appropriate to length–frequency analysis than (L_∞, K, t_0). The biologist actually knows both the shortest and longest observed lengths. In practice l, the mean length of the youngest fish, lies somewhat above the shortest observed length; similarly L lies somewhat below the longest observation. Typically, the mean lengths cluster near L for older fish, while mean lengths for younger fish are more spread out, leaving noticeable peaks. Suppose that the biologist can formulate an opinion on the first and last means, l and L, as well as at least one further mean μ_i for some age a_i ($i \neq 1$ or M). Then these values of l, L, and μ_i can be substituted into (7), and the resulting equation can (in principle) be solved for k. Unfortunately, it is not possible to express analytically a general solution for k in equation (7); however, a few trial values of k will suggest a first estimate. In practice, the biologist may have an opinion about several mean lengths other than l and L. Again, the principle of trying a few k values usually leads to a reasonable first estimate.

In summary, it is not too difficult in many cases to obtain first estimates of l, L, and k which are biologically meaningful. The parameters l and L are based on observations near the shortest and longest lengths in the sample. Information on k then comes from an estimate of one or more means other than the first and last. Conceptually, k represents the fixed fraction by which the annual growth increment is multiplied each year. A choice of k near 1 implies almost uniform growth so that the means are spaced almost evenly between l and L. On the other hand, a choice of k near 0 implies that the annual growth decreases each year to a small fraction of its former size. Consequently, the first increment in mean length is relatively large, while the mean lengths of older fish tend to cluster near L.

By contrast, the significance of L_∞, K, and t_0, is not always obvious and sometimes even deceptive. For example, it is tempting to suppose that L_∞ corresponds roughly to the longest observed length, or a little beyond. However, if k is near 1 and the means are almost evenly spaced, then L_∞ may lie far beyond the observed range. If k is near 0, then L_∞ is approximately equal to L by (8). Since L is the highest *mean* length, it lies below the longest observed length, and, consequently, so might L_∞ in this case. There have also been difficulties with the interpretation of K and t_0. As Ricker (1975, p. 221) points out, "it is misleading to refer to K as a growth rate" because K actually measures the exponential rate of approach to asymptotic size. The parameter K does not involve the units of length; a species may grow rapidly in cm/yr and still be characterized by a small value of K. Also, since t_0 is theoretically the age at which fish have length 0, it is difficult to understand biologically why t_0 is not always exactly 0. This problem, of course, is merely an artifact which results from extending the curve beyond the range of the data; however, it is still difficult to grasp intuitively what a reasonable value of t_0 might be for a particular data set.

Some of the problems just cited have led to controversy in the literature. For example, Knight (1968) shows that the interpretation of L_∞ as an asymptotic length may lead to complete nonsense. The point is that the nonsense stems not from the mathematics, but from the biological interpretation commonly placed on the results. The parameters l, L, and k merely summarize observed facts about the data. By contrast, L_∞, K, and t_0 have been used as indicators of fundamental biological characteristics of the fish. Such interpretations are almost always purely speculative; they may, as Knight points out, be wrong.

In this description of von Bertalanffy growth, emphasis has so far been placed on the mean length for each age. Of course, fish of the same age do not always have the same length; if they did, length–frequency analysis would be trivial. Associated with each mean length μ_i is a standard deviation in length σ_i. Just as the means might conform to a growth relationship, so also the standard deviations might be prescribed by some rule. For example, the standard deviations might be a linear function of the means; that is,

$$(11) \quad \sigma_i = s + (S - s)\frac{\mu_i - l}{L - l}; \qquad i = 1, \ldots, M;$$

where s and S are the standard deviations σ_1 and σ_M for ages a_1 and a_M, respectively. Alternatively, the σ's might be a linear function of the ages; that is,

$$(12) \quad \sigma_i = s + (S - s)\frac{i - 1}{M - 1}; \qquad i = 1, \ldots, M.$$

A special case of both (11) and (12), obtained when $s = S$, is the situation of constant standard deviation

$$(13) \quad \sigma_i = s; \qquad i = 1, \ldots, M.$$

Notice that (7) and (11) taken together imply that

$$(14) \quad \sigma_i = s + (S - s)\frac{1 - k^{i-1}}{1 - k^{M-1}}; \qquad i = 1, \ldots, M.$$

Consequently, in this case, the parameters s, S, and k play a role for the σ's similar to that of l, L, and k for the μ's. In analogy with the means, the standard deviations lie between s and S in such a way that the gap between two successive σ's shrinks each year to the fraction k of its former size. If $k = 1$, the σ's are equally spaced between s and S as described by (12).

In summary, there is a logical organization to the possibilities (11) to (14) for the σ's. Equation (13) is a special case of (12), which, in turn, is a special case of (14). Also, (14) is equivalent to (11) when the means conform to von Bertalanffy growth.

The parameters s and S are not completely analogous to l and L in one significant respect. While it is always true that $l < L$, it may happen as in (13) that $s = S$, or even $s > S$. Many factors may contribute to size variation among fish of one age. If these factors accumulate so that shorter fish fall farther behind and longer fish tend to do better, then it is reasonable that $s < S$. On the other hand, it may happen that younger fish experience considerable variability in growth rate, while older fish tend to reach a limiting size. In this case, as the animals grow older, the small ones tend to catch up, rather like children approaching adulthood. Consequently, $s > S$ because an initially large size range narrows with age. The special case $s = S$ might occur when the tendencies to each extreme just balance.

Length–Frequency Statistics

To describe the statistics of length–frequency sampling, it is necessary to extend the notation of the previous section. In a population with M age classes, let μ_i, σ_i, and π_i be, respectively, the mean length, standard deviation in length, and percentage (or fraction) of fish at age a_i ($i = 1, \ldots, M$). Suppose that fish are sampled randomly from this population and that the length of each fish is determined to lie in one of the N intervals

$$(x_j - w/2, x_j + w/2); \qquad j = 1, \ldots, N;$$

where

$$x_j = x_1 + (j - 1)w$$

is the midpoint of the jth interval and every interval has width w.

Assume that fish lengths are distributed normally in each age-group. Then, given that a fish has age a_i, the probability that its length lies in the jth interval is

$$(15) \quad q_{ij} = \frac{1}{\sigma_i\sqrt{2\pi}}\int_{x_j-w/2}^{x_j+w/2} \exp\left[-\frac{1}{2}\left(\frac{x - \mu_i}{\sigma_i}\right)^2\right]dx.$$

Here the integral (15) defines a section of area under a normal curve. Details for its numerical calculation are given in Appendix C. Suppose that a total frequency f of fish are sampled. Then the expected frequency of fish having length in the jth interval is

$$(16) \quad f_j = f\sum_{i=1}^{M} \pi_i q_{ij}.$$

TABLE 1. Notation summary in logical order.

Classification	Notation	Description
Age descriptors	i	Index for age
	a_i	ith age
	M	Number of age-classes
Length descriptors	j	Index for length
	x_j	Midpoint of jth interval
	w	Width of each interval
	N	Number of length intervals
Population parameters	μ_i	Mean length at age a_i
	σ_i	Standard deviation in length at age a_i
	π_i	Fraction (%) of fish at age a_i
	l, L, k	von Bertalanffy parameters for μ's
	s, S	Parameters for σ's
	P	Total number of population parameters
Sample size	f	Number of fish sampled
Observations	$\hat{f}_j$	Observed frequency in jth interval
	r	Small number of observations (integer)
	N_r	Number of intervals with $\hat{f}_j \geq r$
	$\hat{g}_r$	Observed total from intervals with $\hat{f}_j \geq r$
Expectations	q_{ij}	Probability of jth length, given age a_i
	f_j	Expected frequency in jth interval
	g_r	Expected total from intervals with $\hat{f}_j \geq r$
Measure of closeness	A	Separation statistic
Measure of fit	B_r	χ^2 statistic
	D_r	df

In this sum the product π_i times q_{ij} represents the overall probability that a fish has both age a_i (probability π_i) and a length in the jth interval (probability q_{ij}). Since a fish with specified length must come from one of the age-groups, these probabilities are then summed over the ages to give the expected probability for each length interval. In other words, (16) merely states that the expected number of fish in each length interval is the sum of expected numbers broken down by age.

When a sample is taken, of course, the observed frequency of fish with lengths in the jth interval (call it $\hat{f}_j$) does not, in general, equal the expected frequency (f_j). This is true because the observation $\hat{f}_j$ includes statistical sampling error. In practice, since the population parameters (such as μ's, σ's, and π's) are not known, the expected frequencies are also unknown. The problem is to determine a set of population parameters which would lead to expected frequencies as close as possible to those observed. This requires a criterion for "closeness." In this paper, closeness is measured by the separation statistic

$$(17) \quad A = 2 \sum_{j=1}^{N} \hat{f}_j \log(\hat{f}_j / f_j).$$

The expected frequencies are said to be as close as possible to the observations when A is minimal. The significance of this particular criterion will be discussed shortly. It has been suggested previously for this application by Macdonald and Pitcher (1979), who refer to A as "twice the Kullback minimum discrimination information statistic."[1] For reference, the notation used here is summarized in Table 1.

To understand exactly how this criterion might be applied, notice from (15) that q_{ij} depends on μ_i and σ_i; in symbols,

$$q_{ij} = q_{ij}(\mu_i, \sigma_i).$$

Consequently, the expected frequency f_j depends on the μ's and σ's, as well as the π's. In symbols, from (16),

$$f_j(\mu\text{'s}, \sigma\text{'s}, \pi\text{'s}) = f \sum_{i=1}^{M} \pi_i q_{ij}(\mu_i, \sigma_i).$$

Substituting this result in (17) gives

$$A(\mu\text{'s}, \sigma\text{'s}, \pi\text{'s}) = 2 \sum_{j=1}^{N} \hat{f}_j \log[\hat{f}_j / f_j(\mu\text{'s}, \sigma\text{'s}, \pi\text{'s})].$$

In short, if a set of observed frequencies f_j which total f are given, that is,

$$(18) \quad \sum_{j=1}^{N} \hat{f}_j = f,$$

---

[1] There is no clear precedent for a name for A. Rao (1973; p. 352) refers to a similar statistic as the "Kullback–Liebler separator." Macdonald and Pitcher get their reference to $A/2$ from Kullback (1959). The reason for relating A to "separation" is given in Property 3 later. Alternatively one can speak of "information," or even "entropy." The factor of 2 in (17) makes A approximately a χ^2 statistic. See Appendix B.

then the equations (15)–(17) show explicitly how to calculate A from a given set of population parameters. The problem is to locate a particular set of parameters which minimizes A.

So far, A has been described as a function of the μ's, σ's, and π's. If, however, the means lie on a von Bertalanffy curve (7) determined by l, L, and k, then A can be considered a function of the von Bertalanffy parameters, that is,

$$A = A(l, L, k, \sigma\text{'s}, \pi\text{'s}).$$

Similarly, if the standard deviations depend linearly on the means, following (11) with parameters s and S, then

$$A = A(\mu\text{'s}, s, S, \pi\text{'s}).$$

If both the von Bertalanffy curve (7) and the linearity relation (11) apply, then

$$A = A(l, L, k, s, S, \pi\text{'s}).$$

In short, A is always a function of the population parameters, but the parameters of interest depend on whether or not the means and standard deviations conform to some growth law. If so, then the parameters of the growth law become fundamental. In this way, as stated in the introduction, the previously separate issues of growth and length–frequency analysis become one.

In a typical length–frequency data set, there are length intervals where the observed frequency is small (say, 0, 1, 2, 3, or 4). Some notation is needed to discuss these intervals in a systematic way. For a small number r, let N_r be the number of intervals on which $\hat{f}_j \geq r$. Also let $\hat{g}_r$ be the total number of observations associated with these N_r intervals. In symbols, define

$$(19) \quad g_r = \sum_{j=1}^{N}{}^{(r)} \hat{f}_j,$$

where $\sum^{(r)}$ refers to the sum over those values of j for which $\hat{f}_j > r$. Similarly, let

$$(20) \quad g_r = \sum_{j=1}^{N}{}^{(r)} f_j$$

represent the total expected frequency associated with intervals where $\hat{f}_j > r$.

Notice from (18) and (19) that $\hat{g}_1 = f$ because $\sum^{(1)} \hat{f}_j$ represents the sum of all nonzero frequencies, that is, the total number of observations. By contrast, from the definition (16), the expected frequencies are never zero, inside or outside the observed length range. Consequently, the total expected frequency on intervals where $\hat{f}_j > 1$ is smaller than f; that is, $g_1 < f$. This shows that

$$(21) \quad \sum_{j=1}^{N}{}^{(1)} f_j \leq \sum_{j=1}^{N}{}^{(1)} \hat{f}_j = f,$$

where, ordinarily, the inequality is strict.

To understand the significance of the "minimum A" criterion, the user should be aware of three basic properties of A. These can now be described, with the aid of the notation just defined.

PROPERTY 1. The "minimum A" criterion leads to exactly the same answer as maximum likelihood. However, it is numerically preferable to use A as the criterion, rather than likelihood or log-likelihood.

This should reassure the reader unfamiliar with the separation statistic A. He is actually finding the more familiar maximum likelihood estimates. It turns out that A is closely related to log-likelihood, except for a constant which can be large. This large constant can sometimes mask the behavior of the log-likelihood function, especially when computer precision is limited. Consequently, A tends to be more sensitive to the parameters. Details are given in Appendix B.

PROPERTY 2. In calculating A, one can omit intervals for which there are no observations. In other words, (17) can be replaced by

$$(22) \quad A = 2 \sum_{j=1}^{N}{}^{(1)} \hat{f}_j \log (\hat{f}_j / f_j).$$

The reader may already have noticed (and even been disturbed by) the fact that (17) is not defined if $\hat{f}_j = 0$ for some j, since then the logarithm is not defined. However, in the limiting senses as $\hat{f}_j$ tends to zero, the jth term of A tends to zero. (Recall that f_j is never zero.) This justifies (22). In principle, A can be considered an infinite sum over all possible length intervals; however, the only nonzero terms in the sum are those with $\hat{f}_j > 0$. It follows that A does not depend on how the observed fish lengths are grouped, except through the original choice of intervals. This fact has practical significance, as discussed later in the examples.

PROPERTY 3. The separation statistic A is always positive or zero when condition (21) is true. In fact, A is zero only if

$$(23) \quad f_j = \hat{f}_j$$

for every j.

The fact that A is positive justifies calling it a "separation," particularly since the separation is zero only if expected and observed frequencies agree completely. The inequality $A \geq 0$, which follows from (21), is one of the basic inequalities of information theory. It is proved, for example, by Rao (1973, p. 59). This inequality is not immediately obvious because A contains terms both positive (when $\hat{f}_j > f_j$) and negative (when $\hat{f}_j < f_j$). Notice particularly that, if (23) holds, then $A = 0$ because $\log 1 = 0$. In practice, the predicted frequencies do not exactly equal the observations, and the minimum value of A is positive.

Criteria for Testing the Fit

In length–frequency analysis, the separation statistic A (or, for that matter, any reasonable fitting criterion) is a distinctly nonlinear function of the population parameters. Unlike linear estimation, such as ordinary linear regression, nonlinear estimation problems do not always have the convenient property that there is only one solution. In fact, length–frequency analysis typically leads to many solutions. By analogy, one can think of the fact in elementary algebra that the simplest nonlinear equation, a quadratic, usually has two solutions, while a linear equation has only one. In practice, it is often possible to reject one quadratic solution on physical grounds. For example, one solution might be negative when the solution of interest is positive. However, there is no general theory about which solution is right; the choice depends on the situation. Similarly in length–frequency analysis, the user must select among competing solutions, according to his knowledge of the situation. No general theory will make that choice.

A related problem is that the user may be unsure of the correct model for his data. He may not know precisely how many age classes there are, or what sort of structure (if any) to impose on the means and standard deviations. Often a diversity of different models will fit the data rather well. This problem is typical for length–frequency analysis; the data can be almost too easy to fit.

A simple criterion for selecting among various possibilities would be to pick the one which gives the lowest minimum value of the statistic *A*. Unfortunately, as examples given later illustrate, this may result in a poor choice. Generally (although not necessarily), the greater the number of parameters in a model, the lower the minimum value of *A*. This consideration suggests the usual application of a χ^2 test in which the number of degrees of freedom decreases as the number of estimated parameters increases. Such a test is based on a theorem of statistics, given precisely by Cramér (1946, section 30.3). It states that a certain statistic, evaluated at the maximum likelihood estimates, has asymptotically (for large sample size) a χ^2 distribution.

One way to construct a χ^2 statistic for this problem is to select a small value of *r* (say 1, 2, 3, or 4), and then lump together all those length intervals on which $\hat{f}_j < r$. The corresponding statistic, in the notation of (19)–(20) above, is then

$$(24)\quad B_r = \sum_{j=1}^{N} {}^{(r)} \frac{(\hat{f}_j - f_j)^2}{f_j} + \frac{(g_r - \hat{g}_r)^2}{f - g_r}.$$

Here B_r is associated with $N_r + 1$ length groups, namely the N_r intervals where $\hat{f}_j \geq r$ and one further group of intervals with $f - \hat{g}_r$ observations. This suggests a number of degrees of freedom

$$(25)\quad D_r = N_r - P,$$

where *P* is the number of parameters estimated.

Unfortunately, Cramér's theorem cannot be applied directly to the statistic B_r for two reasons. First, except when $r = 1$, the groups of intervals used in calculating B_r differ from those used in obtaining the likelihood estimates (by minimizing *A*). Second, the method of selecting groups of intervals for B_r depends directly on the observed frequencies f_j.[2] In fact, the distribution of B_r is simply not known in general. It seems reasonable to conjecture that it might be approximately χ^2 distributed, at least for values of *r* large enough to guarantee several observations in every length group (say, $r = 3$ or 4). We do not attempt here to develop a complete mathematical theory for the use of B_r. Instead, we take a strictly pragmatic approach, as described in the next paragraph.

For a given model, we first compute the required parameter estimates by minimizing *A*. This avoids any arbitrary grouping of the data, other than the initial choice of length intervals, at least as far as the estimates are concerned. (See Property 2 for *A*, and the subsequent discussion, above.) Once the estimates are known, we then calculate B_r for several values of *r* and compute the corresponding χ^2 percentage levels for D_r degrees of freedom. (The necessary formulas are given in Appendix C). In comparing solutions obtained from various models, where each model may have more than one minimum point, we simply rank the results according to the χ^2 percentage levels. In this way, we can explore two questions: (1) are the rankings consistent for various values of *r*, and (2) do the highest ranked choices appear most reasonable biologically? The answers suggest that the statistic B_r can be a useful guide, but not a final criterion, for sorting out multiple solutions to a length–frequency estimation problem.

Appendix D gives details of computer methods used to implement all the procedures described in the previous paragraph.

Example 1. Northern Pike

The first data set considered here pertains to Northern pike (*Esox lucius*) from Heming Lake, Manitoba. It was originally presented by Macdonald (1969) and then reanalyzed a decade later by Macdonald and Pitcher (1979). The observed frequency data for this example and the next one are listed in Table 2. These data (previously published in graphical form) are needed for reference in the discussion here.

As Macdonald (1969) describes, the pike sample consists of 523 fish. The length of each is known, as well as an estimate of its age (between 1 and 5 yr) from scale analysis. This allows the results from length–frequency analysis to be compared with a standard. The first line in Table 3 (example 1.1) shows the population parameters, i.e. μ's, σ's, and π's, determined by scale reading. The next line (example 1.2) shows the final results from length–frequency analysis published by Macdonald and Pitcher (1979). Obviously, the agreement between examples 1.1 and 1.2 is quite good.

---

[2] In theory, the method of grouping should depend on the expected frequencies f_j. Since these frequencies are usually unknown, this requirement of Cramér's theorem is almost always violated in practice. The statistic B_r is at least based on a prescribed rule for grouping, not an ad hoc choice of the investigator.

TABLE 2. Observed frequency data for pike and abalone. Frequencies should be read first across and then down. (For example, $\hat{f}_1 = 4$, $\hat{f}_2 = 10$, $\hat{f}_3 = 21$ in the pike data.)

Pike frequencies. $N = 30$, $x_1 = 19$, $x_{30} = 77$, $w = 2$, $f = 523$									
4	10	21	11	14	31	39	70	71	44
42	36	23	22	17	12	12	11	8	3
6	6	3	2	1	1	1	0	1	1
Abalone frequencies. $N = 62$, $x_1 = 8$, $x_{62} = 130$, $w = 2$, $f = 431$									
2	7	7	4	0	0	0	0	0	1
1	4	3	5	7	3	5	1	0	3
0	3	10	3	2	5	9	8	8	15
8	11	13	10	15	13	11	12	14	12
17	14	17	18	20	10	11	10	11	9
7	8	10	5	6	4	4	7	3	4
0	1								

Macdonald and Pitcher arrive at the estimates 1.2 by assuming $\sigma_3 = 4$, $\sigma_4 = 5$, and $\sigma_5 = 6$, as shown. Before minimizing A, they also lump the last six frequencies (1, 1, 1, 0, 1, 1 in Table 2) to obtain a final group of five fish. They argue (p. 991) that this makes the model more robust to nonnormality. Nevertheless, it should be recognized that lumping constitutes an ad hoc decision with regard to the data. One could, instead, lump the last four or five frequencies into a single group. A consequence of this procedure is that the lumped data set does not distinguish between five pike with lengths 67 cm and five pike with respective lengths 67, 69, 71, 75, and 77 cm, as observed. Example 1.3 (Table 3) shows the estimates that are obtained without lumping the data. Not surprisingly, μ_5 turns out to be larger. In fact, because of the fixed standard deviations σ_3, σ_4, and σ_5, the last three means all turn out to be larger in example 1.3 than in 1.2. This shift results in an increase (from 49.5 to 55.6%) in the apparent proportion of age 2 fish.

Example 1.4 (Table 3) shows a minimum point for A obtained without lumping the data, but with the assumption that the standard deviations are linear on the means, as in (11). Notice that, like solution 1.2 of Macdonald and Pitcher (1979), example 1.4 also gives reasonable agreement with the results from scale analysis (example 1.1). The interesting difference is that 1.4 is obtained (i) without assuming explicit values for σ_3, σ_4, and σ_5 and (ii) without ad hoc lumping of the data. Only the linearity relation (11) is assumed. Example 1.5 shows a similar result based on the assumption that the standard deviations increase linearly with age, as in (12). Comparison between examples 1.4 and 1.5 shows that the two linearity assumptions (11) and (12) lead to very nearly the same result.

This discussion is not intended as criticism of solution 1.2 obtained by Macdonald and Pitcher (1979), but rather as an illustration of one more point of view. Part of their approach consists in lumping the data, as deemed appropriate, for robustness. Our approach is to avoid decisions in regard to the data as discussed earlier in connection with Property 2 for A. Instead, we put decisions on structure directly into hypotheses of the model. We utilize data lumping later, only as a tool for assessing the fit. A useful outgrowth of our approach in this case is that we need make no assumptions on particular values for the σ's. Our unknown standard deviations are s and S. In Macdonald and Pitcher's solution 1.2, the unknown deviations are σ_1 and σ_2, while σ_3, σ_4, and σ_5 are presumed known.[3]

Some assumption about standard deviations is certainly necessary for this problem. Examples 1.6 and 1.7 illustrate two minima for A in which there is no restriction on the σ's. (Incidentally, these examples show how A can have more than one local minimum.) Both solutions exhibit a peculiar feature: one of the standard deviations turns out extremely small. In each case an entire age-class is used to explain some minor amount of noise in the data. For instance, in example 1.6 the fifth age-class consists entirely of the two observations which comprise the small extreme right-hand mode of the histogram of observed frequencies shown in Fig. 1. From that point of view, example 1.6 would be an excellent fit if indeed the final mode comprised a whole age-group. In fact, both examples 1.6 and 1.7 suggest that, without some structure imposed on the standard deviations, the analysis points to a solution comprising only four groups with significant numbers of fish. Example 1.8 shows the solution obtained with this assumption, $M = 4$. Aside from components with minor numbers of fish, examples 1.6 to 1.8 look much the same. Biologically, example 1.8 is very reasonable if the combined group of age 4's and 5's is regarded as a single group of undistinguishable fish.

It is well known (see, for example, Macdonald and

---

[3] While the final draft of this paper was in revision, we discussed these results with Prof Macdonald and received a letter in reply showing another interesting result obtained with log-normal components. Certainly, no single approach can be considered definitive. Ordinarily, the practitioner will try several approaches to discover that which best suits his knowledge about the biological background for the data. In making a final decision, the χ^2 analysis given here may prove useful.

TABLE 3. Examples of means, standard deviations, and percentages for various fits to the pike data. All examples except numbers 1.1 and 1.2 correspond to minima for *A*.

Example no.	Mean lengths (cm)					Standard deviations (cm)					Percentages (as fractions)					
	μ_1	μ_2	μ_3	μ_4	μ_5	σ_1	σ_2	σ_3	σ_4	σ_5	π_1	π_2	π_3	π_4	π_5	*A*
1.1[a]	23.33	33.09	41.27	51.24	61.32	2.44	3.00	4.27	5.08	7.07	0.105	0.465	0.298	0.090	0.042	21.28
1.2[b]	22.50	33.10	40.04	48.57	60.02	1.92	3.40	4.00	5.00	6.00	0.085	0.495	0.238	0.128	0.054	19.70
1.3	22.66	33.73	42.13	52.58	65.15	1.80	3.63	4.00	5.00	6.00	0.082	0.559	0.225	0.110	0.024	17.14
1.4	22.67	33.44	40.73	51.27	62.77	1.80	3.53	4.70	6.40	8.24	0.082	0.498	0.265	0.128	0.027	16.20
1.5	22.68	33.29	39.77	50.58	62.10	1.81	3.44	5.07	6.70	8.33	0.083	0.448	0.306	0.134	0.029	16.16
1.6	22.65	33.46	40.48	52.90	76.02	1.79	3.54	5.51	7.78	0.49	0.082	0.469	0.310	0.136	0.003	13.29
1.7	22.74	33.46	40.11	41.77	50.98	1.84	3.37	0.65	5.90	10.11	0.084	0.484	0.034	0.241	0.157	14.98
1.8	22.61	33.65	41.04	51.59	—	1.76	3.67	5.92	9.85	—	0.080	0.490	0.279	0.151	—	16.84

[a] True results from scale reading, published by Macdonald (1969).
[b] Length–frequency solution published by Macdonald and Pitcher (1979).

Pitcher 1979, p. 991) that solutions like 1.6 and 1.7 with standard deviations less than the interval width may be meaningless. The question is, how can they be avoided? For all the examples in Table 3, the values of *A* are lowest by far in examples 1.6 and 1.7. Search algorithms to minimize *A* will try to reach these points. Ad hoc constraints, such as lower bounds on the σ's, may simply result in a solution tight on the constraints as the algorithm seeks the low point. In a biological context, it may be more appropriate to invoke a general hypothesis, such as linearity of the σ's, to avoid unreasonable minima. In other contexts, such as the analysis of physical X-ray spectra, this hypothesis may be completely inappropriate. These remarks illustrate a general point of this paper: the structure of the parameters often distinguishes biological applications of mixture analysis from applications in other fields. The pike example underscores the importance of the σ's. In practice, the biologist may be far more interested in the π's and μ's than he is in the σ's, yet the structure of the σ's may determine the solution he finds.

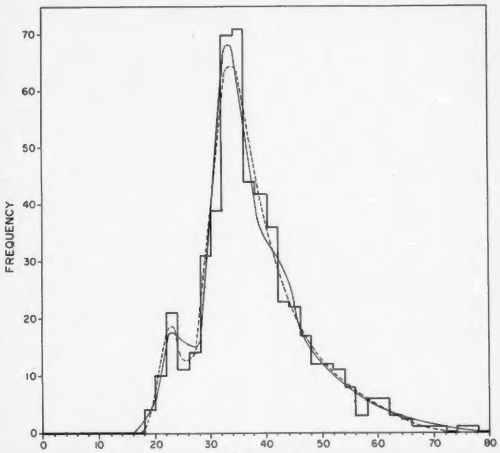


FIG. 1. Length–frequency histogram and two curves of expected frequencies for the pike data. The solid curve pertains to example 1.1, and the broken curve to example 1.4.

All examples in Table 3 lead to a good match between expected and observed frequencies. Figure 1 shows the expected frequency curves for examples 1.1 (solid curve) and 1.4 (broken curve) in relation to the observed frequency histogram. Visually, both curves are close to each other and to the histogram. Similarly, the other examples in Table 3 also correspond to reasonable fits from the point of view of matching the observations, even though the values of *A* vary considerably among the examples. This illustrates the multiplicity problem discussed earlier: the data are almost too easy to fit.

Table 4 shows the results of a χ^2 analysis applied to solutions 1.3 to 1.8 from Table 3. In each case, both the statistic B_r and the corresponding χ^2 level are calculated for $r = 1, 2, 3$, and 4. The resulting χ^2 levels are then used to rank the various solutions. Table 5 lists the rankings obtained in this way. As described earlier, the theoretical basis for this process may be incomplete, but the results are interesting. Rankings are identical with $r = 2$ or 3, and very similar with $r = 4$. For all three values, $r = 2$, 3, or 4, the highest ranked solutions are the most biologically reasonable ones, namely 1.4 and 1.5 in which the σ's increase linearly. The two lowest ranked solutions, 1.6 and 1.7, are those in which one of σ's is unacceptably small. Examples 1.8 (with only four distinguishable age-groups) and 1.3 (with three prescribed σ's) are ranked in the middle. In short, for $r = 2$, 3, or 4, the χ^2 rankings correspond well with biological validity in the results.

When $r = 1$, the χ^2 rankings are less meaningful biologically because the unacceptable solution 1.6 ranks essentially the same as the acceptable solutions 1.4 and 1.5. Table 4 shows χ^2 levels for these three cases which are almost identical (65.1, 65.4, 65.5%) when $r = 1$.

TABLE 4. Quantities for assessing the fit of examples in Table 3. Examples 1.1 and 1.2 are excluded because they do not correspond to minima for A.

Example no.	P	D_r				A	B_r				χ^2 level (%)			
		$r = 1$	$r = 2$	$r = 3$	$r = 4$		$r = 1$	$r = 2$	$r = 3$	$r = 4$	$r = 1$	$r = 2$	$r = 3$	$r = 4$
1.3	11	18	13	12	10	17.14	16.65	12.24	12.22	11.08	54.8	50.8	42.8	35.1
1.4	11	18	13	12	10	16.20	15.10	11.39	11.38	10.38	65.5	57.8	49.7	40.8
1.5	11	18	13	12	10	16.16	15.16	11.34	11.34	10.43	65.1	58.2	50.0	40.4
1.6	14	15	10	9	7	13.29	12.33	11.16	11.16	10.18	65.4	34.5	26.5	17.8
1.7	14	15	10	9	7	14.98	14.72	9.81	9.81	9.26	47.1	45.7	36.6	23.4
1.8	11	18	13	12	10	16.84	16.73	11.70	11.70	11.20	54.2	55.2	47.0	34.4

This difficulty confirms expectations about problems with a χ^2 analysis when $r = 1$. The theory suggests that B_1 simply may not be χ^2 distributed because there may be too few observations in some length-groups.

A related comment applies to the statistic A. For theoretical reasons given in Appendix B, the statistic A is approximately equal to B_1. (See also footnote 1.) This approximation is evident in the examples of Table 4. However, in view of the problems with B_1, it may be wrong to suppose that A is χ^2 distributed. This difficulty underlies the approach taken here whereby the "minimum A" criterion is used to obtain parameter estimates and then the statistic B_r $(r \geq 2)$ is used to rank various proposed solutions. In practice, it appears advisable to check several values of r to see how the rankings are affected.

Example 2. Northern Abalone

The second data set considered here pertains to Northern abalone (*Haliotis kamtschatkana*) from the Queen Charlotte Islands, British Columbia.

It appears, along with numerous other data, in the report by Breen and Adkins (1979). The particular abalone population considered here is a composite associated with all *Nereocystis* communities in the sampled region. (See Fig. 54 of the report.) There are two minor modifications of the published data. Three large animals, deemed by P. A. Breen (personal communication) to lie in a separate group from all the rest, are omitted. Also, to reduce computation, the frequencies are grouped in 2-mm intervals, rather than 1-mm as reported. This grouping probably reflects realistic limits in determining abalone size (P. A. Breen personal communication). The data, so revised, are listed in Table 2.

TABLE 5. Example numbers placed in rank order by the χ^2 levels in Table 4. For each value of r, the six examples are ranked highest to lowest, left to right.

r	Rank order					
	1	2	3	4	5	6
1	1.4	1.6	1.5	1.3	1.8	1.7
2	1.5	1.4	1.8	1.3	1.7	1.6
3	1.5	1.4	1.8	1.3	1.7	1.6
4	1.4	1.5	1.3	1.8	1.7	1.6

There is no known independent method of aging this species. Consequently, a standard, like example 1.1 for the pike data, is not available for comparison with results from length–frequency analysis. The precise number of age-classes is not even known, but it is believed to be much larger than 5. The length–frequency histogram in Fig. 2, with its many modes, suggests that this might be true. In spite of these problems, some independent information is available on growth. Abalone have been tagged and recovered 1 yr later to measure annual growth (Quayle 1971). Using a Walford plot of these size data, one can estimate L_∞ and k. (See Ricker 1975.) In this way Breen (1980) obtains a value of L_∞ equal to 128.9 mm and k equal to 0.766.

In view of this evidence, it is reasonable to investigate the results of length–frequency analysis based on von Bertalanffy growth. As a start, consider some possible age-groups suggested by modes in the histogram

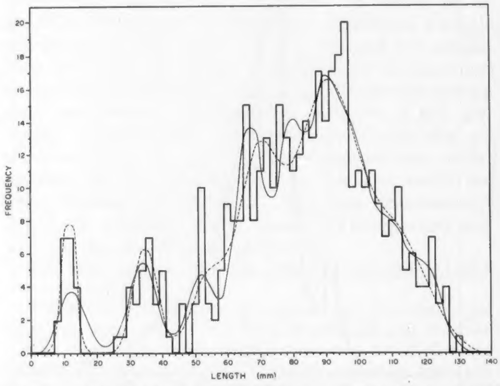


FIG. 2. Length–frequency histogram and two curves of expected frequencies for the abalone data. The solid curve pertains to example 2.1, and the broken curve to example 2.4.

TABLE 6. Examples of means (mm), standard deviations (mm), and percentages (as fractions) for various fits to the abalone data. All examples correspond to minima for A.

Example no.	Quantity	$i = 1$	$i = 2$	$i = 3$	$i = 4$	$i = 5$	$i = 6$	$i = 7$	$i = 8$	$i = 9$	$i = 10$	$i = 11$	A
	Modes[a]	11	36	52	66	76	86	96	102	112	?	122	
	Est. μ's[b]	11	31.7	49.4	64.3	77.1	87.9	97.1	104.9	111.5	117.2	122	
2.1	μ's	12.38	33.90	51.84	66.80	79.27	89.67	98.34	105.56	111.59	116.61	120.80	66.13
	σ's	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	4.25	
	π's	0.046	0.069	0.059	0.170	0.163	0.182	0.135	0.051	0.067	0	0.058	
2.2	μ's	11.29	34.76	53.17	67.61	78.93	87.81	94.78	100.25	104.53			51.33
	σ's	2.05	3.35	4.65	5.95	7.25	8.55	9.85	11.15	12.45			
	π's	0.047	0.068	0.058	0.178	0.071	0.241	0.015	0.032	0.290			
2.3	μ's	11.28	34.98	53.75	68.60	80.36	89.67	97.03	102.87	107.48	111.14		46.55
	σ's	1.84	3.66	5.10	6.24	7.14	7.86	8.42	8.87	9.22	9.50		
	π's	0.046	0.069	0.063	0.200	0.074	0.274	0.089	0	0	0.185		
2.4	μ's	11.29	34.87	53.87	69.19	81.53	91.48	110.64					46.26
	σ's	1.83	3.66	5.14	6.33	7.29	8.06	9.55					
	π's	0.046	0.069	0.065	0.209	0.084	0.329	0.198					

[a] From Fig. 2.

[b] From a von Bertalanffy curve with $l = 11$, $L = 122$, $k = 0.85$ (or $L_\infty = 149.2$, $K = 0.1625$, $t_0 = 0.5288$).

of Fig. 2. Table 6 lists a set of 10 major modes selected by eye. The nine gaps between them (25, 16, 14, 10, 10, 10, 6, 10, 10 mm, respectively) appear to shrink somewhat in von Bertalanffy fashion, but with a problem toward the end. One possible way to deal with this problem is to add an extra age-class, thus making two small gaps in place of one large one at the end. For instance, the parameters $l = 11$ mm, $L = 122$ mm, and $k = 0.85$ with 11 age-classes generate a set of means reasonably close to the observed modes, as shown in Table 6. Here l and L correspond to the first and last observed modal lengths, and k is found by trial and error to give reasonable approximations for the remaining modes. In this way, the histogram itself suggests first estimates for l, L, and k. Incidentally, the corresponding parameters, $L_\infty = 149.2$ mm, $K = 0.1625$, and $t_0 = 0.5288$ are not amenable to such simple intuitive motivation. (For example, L_∞ lies well beyond the range of data.) This illustrates the utility of the new von Bertalanffy parameters for length–frequency analysis.

When the eye scans the histogram in Fig. 2 and seeks to identify age-classes with the various modes, it tends to pick out clusters with roughly the same width. Example 2.1 in Table 6 reflects this perception. It is a solution with means on a von Bertalanffy curve, constant standard deviations, and 11 age-classes (in accord with the last paragraph). The solid curve in Fig. 2 represents the predicted frequencies for this case. The various age-groups show up distinctly as either modes or bends in the curve. Not surprisingly, in Table 6 the age-class next to the last registers zero percentage of the fish, since it appears as an artifact to accommodate von Bertalanffy growth. In Table 7, the parameters l, L, and k for example 2.1 turn out remarkably close to the initial estimates of 11, 122, and 0.85, respectively. In this case there are 14 parameters (l, L, k, s, and 10 π's), rather than 32 as would be required by an unstructured analysis of 11 age-classes. Unfortunately, in spite of the comparatively low number of parameters, Table 8 shows that the fit is still not good enough. Example 2.1 would be rejected by χ^2 analysis, for each value of r, even at the 5% cutoff level. Apparently, the requirement of constant standard deviations is too restrictive. In fact, Fig. 2 suggests this. For instance, the solid curve fits the first age-class poorly, because the estimated value of σ_1 is too large. This forces the first mode to be too wide and low compared with the corresponding mode on the histogram.

Example 2.2 (Table 6) seeks to overcome this problem by allowing the standard deviations to be linear on the age. The value of A drops, and the first age-class is accommodated much better. However, the σ's now become very large for the older age-classes, because they must increase by a fixed amount from each age to the next. In fact, σ_9 is so large that the ninth age-group accounts for all observations above 104 mm. This suggests a different perception of the histogram in Fig. 2. Perhaps the modes at higher lengths do not correspond to separate age-classes, but are just noise on the descending limb of a single normal curve.

One way to avoid the rapid growth in standard deviations of example 2.2 is to let them be linear on the means, so that the σ's reach a limiting size with the μ's. Example 2.3 illustrates a solution with this assumption. The results in Table 6 are similar to example 2.2, except for the higher age-groups. In particular, example 2.3 contains a final age-group quite isolated from all the rest. It appears that for the abalone population, with its numerous age-groups, the particular linearity assumption, (11) or (12), on the σ's may be important. This contrasts with the situation for pike, where either

TABLE 7. Parameters for the means and standard deviations in the fits to the abalone data cited in Table 6. The standard deviations are either constant (CO), linear on age (LA), or linear on the means (LM).

Example no.	Age-classes	l	L	k	L_∞	K	t_0	σ's	s	S
2.1	11	12.38	120.80	0.8337	141.78	0.1819	0.4978	CO	4.25	—
2.2	9	11.29	104.53	0.7843	120.12	0.2429	0.5936	LA	2.05	12.45
2.3	10	11.28	111.14	0.7916	125.02	0.2337	0.5954	LM	1.84	9.50
2.4	6	11.29	91.48	0.8059	132.78	0.2158	0.5882	LM	1.83	8.06

TABLE 8. Quantities for assessing the fit of examples in Table 6.

Example no.	P	D_r				A	B_r				χ^2 level (%)			
		$r = 1$	$r = 2$	$r = 3$	$r = 4$		$r = 1$	$r = 2$	$r = 3$	$r = 4$	$r = 1$	$r = 2$	$r = 3$	$r = 4$
2.1	14	40	36	34	28	66.13	57.69	54.19	53.02	49.14	3.5	2.6	2.0	0.8
2.2	13	41	37	35	29	51.33	49.02	43.44	42.93	37.83	18.2	21.6	16.8	12.6
2.3	14	40	36	34	28	46.55	43.65	39.46	38.73	34.26	31.9	31.8	26.5	19.2
2.4	12	42	38	36	30	46.26	43.33	39.37	38.62	34.17	41.4	40.8	35.2	27.4

assumption gave similar results. (See examples 1.4 and 1.5.)

Examples 2.2 and 2.3, especially the isolated final age-group of 2.3, suggest a new approach to the analysis. Perhaps the animals grow rapidly at first, forming identifiable age-classes, and then finally growth slows so much that all animals after a certain age appear as a single group. These ideas motivate example 2.4. Here the first six age-classes have means on a von Bertalanffy curve, while the last mean is independent of the rest. The standard deviations, including the last, are presumed linear on the means. There are 12 parameters: l, $L(=\mu_6)$, k, μ_7, s, S, and 6 π's. The predicted frequencies for this case are represented by the broken curve in Fig. 2. It is less undulating than the solid curve (for example 1.1), reflecting fewer age-classes. The general fit, especially for the first two age-classes, is distinctly better than in example 1.1. Notice also that the broken curve descends steadily through the higher modes of the histogram, which are regarded by the statistical model as noise.

Of the four length–frequency solutions given here for the abalone data, example 2.4 is certainly the most attractive. Table 8 shows that even though example 2.4 has the fewest parameters, it gives rise to the lowest minimum value of A. Not surprisingly, then, the χ^2 analysis ranks example 2.4 highest. Incidentally, in this case, unlike the earlier one for pike, the ranking of solutions is the same for $r = 1$, 2, 3, or 4. Finally, Table 7 shows that example 2.4 corresponds to values of L_∞ (132.78 mm) and k (0.8059) which agree reasonably well with the values ($L_\infty = 128.9$ mm, $k = 0.766$) obtained independently from other data by Breen (1980).

These four examples certainly do not exhaust the possibilities for discussion of the abalone data, and they should not be regarded as final biological conclusions. They do, nevertheless, illustrate how growth structure aids the discussion of length–frequency analysis. Notice particularly how these methods assist in determining the number of age-classes. Indeed, part of the abalone analysis involves deciding how many age-classes are discernible. Growth structure appears to be a useful tool in making that decision. Possibilities for future analysis of the abalone data include a parametric description of the π's to reflect mortality and less restrictive descriptions of the μ's and σ's.

Conclusions

The examples in the previous two sections are intended to help the reader develop intuition on the use of growth structure in length–frequency analysis. Although the problem of multiple solutions makes a general theory impossible, the examples do suggest several basic conclusions or guidelines. These are presented here.

1. Because length–frequency analysis may lead to many solutions for the same data set, subjective decisions must often be made on biological grounds. One way to introduce biological opinion is to require that the means and standard deviations conform to an assumed growth model.

2. The χ^2 level associated with the statistic B_r in (22) can be helpful for choosing among competing solutions, although it should not be regarded as definitive. Solution rankings by χ^2 level tend to be consistent for low values of $r > 1$. On purely statistical grounds, the χ^2 analysis may suggest actually rejecting only a few of many competing solutions.

3. Even if the percentages and means are the parameters of greatest interest, the structure of the standard deviations may be the most important feature in determining which solution is obtained. If the σ's are assumed to have linear growth, it is generally more realistic to presume linearity on the means (11), rather than on the ages (12), particularly when the number of age-classes is large.

4. A von Bertalanffy structure for the means is sometimes too restrictive, but it can be useful for suggesting the number of discernible age-classes when that number is unknown. It can also be used to check the results of length–frequency analysis against an independent measure of growth from a tagging study over 1 yr.

5. Reasonable estimates for l, L, and k can often be obtained directly from the length–frequency histogram. By contrast, the corresponding values of L_∞, K, and t_0 may be much less apparent and even misleading.

6. In the minimization process, the parameters l, L, and k tend to be much more stable numerically than L_∞, K, and t_0.

7. Length–frequency analysis tends to lump the final age-classes together if they are in close proximity or contain small percentages of fish. In such cases it may be impossible to distinguish the final ages, and the best approach may be to assume that all fish beyond a certain age comprise a single group.

Acknowledgments

We are grateful to Prof Peter Macdonald for numerous helpful comments on this problem. He sent us an advance copy of his paper with Dr Pitcher, a listing of the programs used in their data analysis, and a sample run. We have been very fortunate to have their published work available as an example for investigating how growth-structured analysis might differ from earlier methods. We are also grateful to Dr Paul Breen for extensive advice regarding the abalone data which he and Bruce Adkins collected in 1978.

ANON. 1964. Handbook of mathematical functions. U.S. National Bureau of Standards, Washington, D.C.

BENYON, P. R. 1976. Remark AS R15, function minimization using a simplex procedure. Appl. Stat. 25: 97.

VON BERTALANFFY, L. 1938. A quantitative theory of organic growth. Hum. Biol. 10: 181–213.

BREEN, P. A. 1980. Measuring the fishing intensity and annual production in the abalone fishery of British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. (In press)

BREEN, P. A., AND B. E. ADKINS. 1979. A survey of abalone populations on the east coast of the Queen Charlotte Islands, August 1978. Fish. Mar. Serv. MS Rep. 1490: 125 p.

CASSIE, R. M. 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshwater Res. 5: 513–522.

CHAMBERS, J. M., AND J. E. ERTEL. 1974. Remark AS R11, a remark on Algorithm AS 47, function minimization using a simplex procedure. Appl. Stat. 23: 250–251.

CRAMÉR, H. 1946. Mathematical methods of statistics. Ninth printing, 1961. Princeton Mathematical Series, vol. 9, Princeton University Press, Princeton, NJ.

FORD, E. 1933. An account of the herring investigations conducted at Plymouth during the years from 1924–1933. J. Mar. Biol. Assoc. U.K. 19: 305–384.

HARDING, J. P. 1949. The use of probability paper for graphical analysis of polymodal frequency distributions. J. Mar. Biol. Assoc. U.K. 28: 141–153.

HASSELBLAD, V. 1966. Estimation of parameters for a mixture of normal distributions. Technometrics 8: 431–444.

HILL, I. D. 1978. Remark AS R28, a remark on Algorithm AS 47, function minimization using a simplex procedure. Appl. Stat. 27: 380–382.

KNIGHT, W. 1968. Asymptotic growth: an example of nonsense disguised as mathematics. J. Fish. Res. Board Can. 25: 1303–1307.

KULLBACK, S. 1959. Information theory and statistics. Wiley, New York. 395 p.

MACDONALD, P. D. M. 1969. FORTRAN programs for statistical estimation of distribution mixtures: some techniques for statistical analysis of length–frequency data. Fish. Res. Board Can. Tech. Rep. 129: 45 p.

MACDONALD, P. D. M., AND T. J. PITCHER. 1979. Age groups from size–frequency data: a versatile and efficient method of analysing distribution mixtures. J. Fish. Res. Board Can. 36: 987–1001.

MCNEW, R. W., AND R. C. SUMMERFELT. 1978. Evaluation of a maximum likelihood estimator for analysis of length–frequency distributions. Trans. Am. Fish. Soc. 107: 730–736.

MELSA, J. L., AND A. P. SAGE. 1973. An introduction to probability and stochastic processes. Prentice-Hall Elec. Eng. Series. Prentice-Hall, Englewood Cliffs, NJ.

NELDER, J. A., AND R. MEAD. 1965. A simplex method for function minimization. Comput. J. 7: 308–313.

O'NEILL, R. 1971. Algorithm AS 47, function minimization using a simplex procedure. Appl. Stat. 20: 338–345.

1974. Corrigendum, Algorithm AS 47, function minimization using a simplex procedure. Appl. Stat. 23: 252.

PEARSON, K. 1894. Contributions to the mathematical theory of evolution. Philos. Trans. R. Soc. London, Ser. A, 185: 71–110.

PETERSEN, C. G. J. 1892. Fiskensbiologiske forhold i Holboek Fjord, 1890–91. Beret. Dan. Biol. St. 1890(91), 1: 121–183.

QUAYLE, D. B. 1971. Growth, morphometry, and breeding in the British Columbia abalone (*Haliotis kamtschatkana* Jonas). Fish. Res. Board Can. Tech. Rep. 279: 84 p.

RAO, C. R. 1973. Linear statistical inference and its applications. 2nd ed. John Wiley and Sons. New York, London, Sydney, Toronto. 625 p.

RICKER, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fish. Res. Board Can. Bull. 191: 382 p.

VAN DER WAERDEN, B. L. 1969. Mathematical statistics. 2nd (English) ed. Springer-Verlag. New York, Heidelberg, Berlin. 367 p.

WALFORD, L. A. 1946. A new graphic method of describing the growth of animals. Biol. Bull. 90: 141–147.

Appendix A. Derivation of Equation (7)

Equation (7), which is an alternative form of the von Bertalanffy growth relationship (2), follows from three assumptions, namely,

(A1) $\mu_{t+2} - \mu_{t+1} = k(\mu_{t+1} - \mu_t)$, (A2) $\mu_1 = l$,

(A3) $\mu_M = L$.

Here, (A1) follows from (3)–(4), and (A2) and (A3) are the definitions of l and L. When $k = 1$, (A1) shows that the means are evenly spaced, as stated in connection with (7). When $k \neq 1$, (A1) can be regarded as a second-order difference equation. It is linear with constant coefficients. The boundary conditions for (A1) are (A2)–(A3).

One possible solution to (A1) is

$$\mu_i = \alpha$$

for some constant α. Another possible solution, as the reader may verify is

$$\mu_i = \beta k^i$$

for some constant β. The theory of difference equations states that any solution to (A1) must be a combination of these two solutions, that is,

(A4) $\mu_i = \alpha + \beta k^i$.

Substituting (A4) in (A2) and (A3) gives

(A5) $l = \alpha + \beta k$,

(A6) $L = \alpha + \beta k^M$.

Equations (A5)–(A6) can be solved for α and β. Substituting the solutions in (A4) gives (7).

Another approach, independent of the one just given, involves showing algebraically that (4)–(6) and (8)–(10) are inverse transformations of each other. It can then be verified that (4)–(6) transforms (2) into (7), and (8)–(10) transforms (7) into (2).

Appendix B. Likelihood, Separation A, and χ^2

The log-likelihood function C associated with the observations $\hat{f}_j$ is given by

(B1) $C = \sum_{j=1}^{N} \hat{f}_j \log (f_j/f)$.

(See, for example, van der Waerden 1969, p. 186). In the sum (B1), the jth term is 0 when $\hat{f}_j = 0$, consequently,

(B2) $C = \sum_{j=1}^{N}{}^{(1)} \hat{f}_j \log (f_j/f)$,

where the special $\sum$ notation is defined in connection with (19). It follows from (B2) and (22) that

(B3) $A = -2C + 2 \sum_{j=1}^{N}{}^{(1)} \hat{f}_j \log (\hat{f}_j/f)$.

In (B3) notice that, while A and C depend on the population parameters, the summation depends only on the observations. As functions of the parameters, then, the separation statistic A is minus twice the log-likelihood plus a constant. This shows that C is a maximum when A is a minimum, and vice versa, as stated in Property 1.

The constant term in (B3) is always negative, and it can be quite large. For example, with the pike data it is −2991.29. In Table 3, the values of A go from about 13 to 21, producing a change in the first decimal place from the smallest to the largest value. By contrast, the values of C for these examples would go roughly from −1506 to −1502, resulting only in a change of the fourth decimal place. This substantiates the claim in Property 1 that, if computer precision is limited, A makes a better objective function than C.

The value of A has the added advantage that it is more meaningful than C because it approximates the χ^2 statistic B_1. If x is near 1, then

(B4) $\log x \cong (x - 1) - \frac{1}{2}(x - 1)^2$.

When A is minimized, the expected frequency f_j is near the observed $\hat{f}_j$, so that the ratio $f_j/\hat{f}_j$ is near 1. It follows from (22) and (B4) that

$$\begin{aligned} A &= -2 \sum_{j=1}^{N}{}^{(1)} \hat{f}_j \log (f_j/\hat{f}_j) \\ &\cong -2 \sum_{j=1}^{N}{}^{(1)} \hat{f}_j \left[\left(\frac{f_j}{\hat{f}_j} - 1\right) - \frac{1}{2}\left(\frac{f_j}{\hat{f}_j} - 1\right)^2\right] \\ &= -2 \sum_{j=1}^{N}{}^{(1)} (\hat{f}_j - f_j) + \sum_{j=1}^{N}{}^{(1)} (f_j - \hat{f}_j)^2/\hat{f}_j \\ &= 2(f - g_1) + \sum_{j=1}^{N}{}^{(1)} (\hat{f}_j - f_j)^2/\hat{f}_j. \end{aligned}$$

In the final sum above, one can substitute f_j for $\hat{f}_j$ in the denominator with the same degree of approximation already used in applying (B4). This gives

(B5) $A \cong 2(f - g_1) + \sum_{j=1}^{N}{}^{(1)} (\hat{f}_j - f_j)^2/f_j$.

The definition (24) for B_1, taken with the fact that $g_1 = f$ (as discussed in connection with (19)), shows that

(B6) $B_1 = (f - g_1) + \sum_{j=1}^{N}{}^{(1)} (f_j - f_j)^2/f_j$.

It follows from (B5)–(B6) that

(B7) $A \cong (f - g_1) + B_1$.

Ordinarily, g_1 (the sum of expected frequencies on intervals with $\hat{f}_j \geq 1$) is close to f at minimum A. Since $f > g_1$, (B7) suggests that A should typically be slightly larger than B_1, as it is in all the examples of Tables 4 and 8.

Appendix C. The Normal Integral and χ^2 Levels

At biological laboratories, the library of mathematical literature is sometimes quite limited. As a result, some readers of this paper may have difficulty locating methods to calculate the normal integral (15) and to determine χ^2 levels associated with the statistic B_r. For convenience, suitable formulas are cited here.

Hasselblad (1966) suggests calculating q_{ij} in (15) by the approximation

(C1) $q_{ij} = \dfrac{w}{\sigma_i\sqrt{2\pi}} \exp \left[-\frac{1}{2}\left(\dfrac{x_j - \mu_i}{\sigma_i}\right)^2\right]$.

Unfortunately, (C1) can be a poor approximation when w is as large as one of the σ's. This is the case in all pike examples, where σ_1 is about 1.8 and w is 2. For the sake of improving (C1), define a function $F(z)$ sequentially as follows:

$$t = (1 + b_0|z|)^{-1},$$

$$u = \sum_{i=1}^{5} b_i t^i,$$

$$v = \frac{1}{\sqrt{2\pi}} \exp(-z^2/2),$$

$$F(z) = \begin{cases} 1 - uv, & z > 0 \\ uv, & z < 0, \end{cases}$$

where the constants b_0 to b_5 are, respectively, 0.2316419, 0.319381530, −0.356563782, 1.781477937, −1.821255978, and 1.330274429. Also define z_+ and z_- by

$$z_{+,-} = \frac{x_j - \mu_i}{\sigma_i} \pm \frac{w}{2\sigma_i}.$$

Then a good approximation to q_{ij} is

$$\text{(C2)} \quad q_{ij} = F(z_+) - F(z_-).$$

It turns out that the approximations (C1) and (C2) are close if w is small compared to σ_i because then the difference $z_+ - z_-$ is small compared to 1. The approximations leading to (C2) come from Anon. (1964).

The calculation of χ^2 levels involves two functions defined for $z > 0$ and an integer n, namely,

$$G_1(z, n) = (z/2)^{n/2} e^{-z/2} \left[1 + \sum_{i=1}^{\infty} \prod_{j=1}^{i} \left(\frac{z}{n + 2j}\right)\right],$$

$$G_2(n) = \begin{cases} \prod_{j=1}^{n/2} j & , n \text{ even} \\ \sqrt{\pi} \prod_{j=1}^{(n+1)/2} (j - \frac{1}{2}), & n \text{ odd} \end{cases}$$

Here the quantity $G_2(n)$ is, in fact, the gamma function calculated at $1 + n/2$. The χ^2 level, H, for the statistic B_r with D_r degrees of freedom, which corresponds to the probability that the statistic has a value B_r or larger, is

$$\text{(C3)} \quad H(B_r, D_r) = 1 - G_1(B_r, D_r)/G_2(D_r).$$

Formula (C3) is used to obtain the final four columns in Tables 4 and 8. The theoretical basis for (C3) is given by Melsa and Sage (1973).

Appendix D. Computer Methods

Numerous algorithms are available for performing a computer search to locate a function minimum. Some require the function's derivatives, and others, called direct search methods, do not. Although derivative-based algorithms are usually more efficient of machine time, direct search methods typically involve less human time because they do not require the user to calculate and program derivatives. In this application, where the function A can depend on the parameters in many different ways, it is particularly inconvenient to produce the appropriate derivatives for each case. Macdonald and Pitcher (1979) employ the Nelder–Mead algorithm, a remarkably simple direct search technique. The idea behind the method was originally formulated by Nelder and Mead (1965). Later, O'Neill (1971) translated it into a computer program. Various corrections have since been suggested by Chambers and Ertel (1974), O'Neill (1974), Benyon (1976), and Hill (1978). All minima reported here were located by this technique.

If a direct search method is used, this paper contains all formulas necessary for writing a computer program to generate results like those in Tables 3–8. For convenience in describing the calculations, let $z_1, \ldots, z_P$ be the P parameters used by the statistical model. Suppose that the first P_1 z's determine the μ's, the next P_2 z's determine the σ's, and the final P_3 z's determine the π's. (Thus $P_1 + P_2 + P_3 = P$.) For example, if the μ's are required to lie on a von Bertalanffy curve, then $P_1 = 3$ with $z_1 = l$, $z_2 = L$, $z_3 = k$. If the μ's are unrestricted, then $P_1 = M$ with $z_i = \mu_i$ for i from 1 to M. Similarly $P_2 = 2$ if the σ's are linear, and $P_2 = M$ if the σ's are unrestricted. In all examples here $P_3 = M - 1$, because the last percentage is determined by the previous ones.

In addition to the direct search algorithm, the user must supply program modules to do the following eight tasks:

1. Calculate the μ's from the first P_1 z's. This might involve (7).
2. Calculate the σ's from the next P_2 z's, as well as the μ's if required. This might involve one of (11)–(14).
3. Calculate the π's from the final P_3 z's.
4. Calculate the f's from the μ's, σ's, and π's. This involves (15)–(16), as well as (C2).
5. Calculate A from the f's and $\hat{f}$'s. This involves (22).
6. Calculate, and add to A, penalty functions which are large at forbidden parameter values, such as $\pi_i < 0$ or $\pi_i > 1$.
7. Given r, calculate B_r and D_r from the f's and $\hat{f}$'s. This involves (19)–(20), (24), and (25).
8. Calculate a χ^2 level H from B_r and D_r. This involves (C3).

These programs should be kept modular so that individual steps, especially 1 and 2 above, can readily be adapted to a particular model.

After these tasks have been programmed, they can be used by two master programs. In the first, the search algorithm calls steps 1–6 to compute A at the point $(z_1 \ldots, z_P)$. This allows the algorithm to explore P-dimensional space and locate a minimum point. The penalty functions (step 6) play an important role in forcing the algorithm to avoid unreasonable parameters. For example, $10^6\pi_i^2$ might be added to A if $\pi_i < 0$ and $10^6(1 - \pi_i)^2$ might be added if $\pi_i > 1$. Other parameters might also be restricted if the user finds it appropriate. The second master program, which is used after a minimum has been located, calls steps 1–4 and 7–8 to determine the relevant χ^2 levels for the minimum point.

Herbivore Diet Affects Fecal Pellet Settling[1]

P. K. BIENFANG

The Oceanic Institute, Waimanalo, HI 96795, USA

BIENFANG, P. K. 1980. Herbivore diet affects fecal pellet settling. Can. J. Fish. Aquat. Sci. 37: 1352–1357.

The sinking rates of fecal pellets produced by herbivorous zooplankton grazing upon phytoplankton stocks composed primarily of diatoms and flagellates were studied and found to be significantly ($P < 0.01$) different. Under diatom diet, fecal pellets of *Calanus* sp. showed ranges of volume and sinking rate of $0.38–2.51 \cdot 10^6\ \mu m^3$ and $70–171\ m \cdot d^{-1}$, respectively; under flagellate-based diet the pellet volumes and sinking rates ranged from 0.42 to $3.52 \cdot 10^6\ \mu m^3$ and 51 to $152\ m \cdot d^{-1}$, respectively. Sinking rates for the two sets of fecal pellets were related to compositional density, and significantly ($P < 0.01$) correlated with the excess density ($\Delta\rho = \rho_p - \rho_{sw}$) between the particle and the fluid. No significant ($P < 0.05$) correlation was found between the sinking rates and volumes of fecal pellets examined in these two sets. Calculated pellet densities (ρ_p) under diatom and flagellate-based nutrition averaged 1.174 and $1.114\ g \cdot cm^{-3}$, and conferred average sinking rates of 123.3 and $87.9\ m \cdot d^{-1}$, respectively. A mathematical treatment of the functional parameters relevant to the vertical flux of excretory materials is presented, and by application of steady-state turnover theory, provides a framework to estimate the downward transfer rate per unit area of any given pellet constituent considered.

Key words: fecal pellets, herbivorous zooplankton, *Calanus*, nutrition, sinking rate, model

BIENFANG, P. K. 1980. Herbivore diet affects fecal pellet settling. Can. J. Fish. Aquat. Sci. 37: 1352–1357.

Les vitesses de plongée de boulettes fécales produites par du zooplancton herbivore broutant à même des stocks de phytoplancton constitués surtout de diatomées et de flagellés ont été étudiées, et nous avons constaté des différences significatives ($P < 0{,}01$) selon le régime. Avec un régime de diatomées, les boulettes fécales de *Calanus* sp. ont un volume et une vitesse de plongée variant de 0,38 à $2{,}51 \cdot 10^6\ \mu m^3$ et de 70 à $171\ m \cdot d^{-1}$ respectivement; avec un régime constitué surtout de flagellés, le volume et les vitesses de plongée des boulettes varient de 0,42 à $3{,}52 \cdot 10^6\ \mu m^3$ et de 51 à $152\ m \cdot d^{-1}$ respectivement. Les vitesses de plongée pour les deux groupes de boulettes fécales sont fonction de leur densité, et il y a corrélation significative ($P < 0{,}01$) avec l'excédent de densité ($\Delta\rho = \rho_p - \rho_{sw}$) entre la particule et le liquide. Nous n'avons pas trouvé de corrélation significative ($P < 0{,}05$) entre les vitesses de plongée et les volumes des boulettes fécales examinées dans ces deux séries. Les densités calculées des boulettes (ρ_p) avec régimes de diatomées et de flagellés sont en moyenne de 1,174 et $1{,}114\ g \cdot cm^{-3}$, et donnent des vitesses moyennes de plongée de 123,3 et $87{,}9\ m \cdot d^{-1}$ respectivement. Nous présentons un traitement mathématique des paramètres fonctionnels pertinents au flux vertical de matériaux d'excrétion. En appliquant la théorie du renouvellement dans un état d'équilibre, ce modèle peut servir à estimer le taux de transfert vers le bas par unité de superficie pour tout constituant de boulettes étudié.

Received May 7, 1979
Accepted May 23, 1980

Reçu le 7 mai 1979
Accepté le 23 mai 1980

SEVERAL studies have suggested the potential importance of zooplankton fecal pellets to the vertical transfer of material in aquatic environments (Osterberg et al. 1963; Smayda 1969, 1971; Lowman et al. 1971; Schrader 1971; Fowler and Small 1972; Cherry et al. 1975; Ferrante and Parker 1977; Turner 1977; Honjo and Roman 1978; Small et al. 1979). Both size and composition influence particle settling rates in fluid (e.g. Hutchinson 1967); compared to phytoplankton, the larger size and at least partially nonviable composition (Smayda 1971; Porter 1975; Honjo and Roman 1978; Turner 1977; Small et al. 1979) of fecal pellets result in higher sinking for these parcels.

The 1978 controlled ecosystem population experiment (CEPEX) (FOODWEB I), conducted in the Saanich Inlet of British Columbia, addressed a range of questions concerning trophic relationships in marine food webs. Manipulation of the chemical and physical environments within the large ($1300\ m^3$) enclosures produced two distinct phytoplankton communities, one dominated by diatoms and the other by flagellates. This markedly different taxonomic composition at the primary trophic level provided the opportunity to evaluate

---

[1] Contribution 157, Oceanic Institute.

Printed in Canada (J5624)
Imprimé au Canada (J5624)

TABLE 1. Summary of data for fecal pellet length, width, volume, sinking rate, excess density ($\Delta\rho = \rho_p - \rho_{sw}$), and pellet density ($\rho_p$) under diatom and flagellate nutrition. Data give the mean ($\bar{x}$), standard error (SE), and percent SE/$\bar{x}$ for each set of pellets.

		Fecal pellets on a diatom diet			Fecal pellets on a flagellate diet		
Parameter	Units	$\bar{x}$	SE	10^2 (SE/$\bar{x}$)	$\bar{x}$	SE	10^2 (SE/$\bar{x}$)
Length	μm	333	14.4	4.3%	398	22.1	5.6%
Width	μm	61	2.1	3.4%	65	2.3	3.5%
Volume	$10^6\ \mu m^3$	1.10	0.09	8.2%	1.25	0.11	9.2%
Sinking rate	$m \cdot d^{-1}$	123.3	5.56	4.5%	87.9	7.2	8.1%
$\Delta\rho$	$g \cdot cm^{-3}$	0.156	0.009	5.6%	0.093	0.009	10.1%
ρ_p	$g \cdot cm^{-3}$	1.174	0.009	0.75%	1.114	0.009	0.8%

fecal pellet sinking rates and to test the hypothesis that the sinking rates of fecal pellets of herbivorous zooplankton feeding on diatom and flagellate populations may be different. This could result from density differences arising from either varying amounts of contained silica ($\rho = 2.6\ g \cdot cm^{-3}$), or differences in packing of fecal material. Measured sinking rate and size data were employed in a rearranged hydrodynamic equation for circular cylinders to compare the densities (ρ_p) of fecal pellets produced under the two types of nutrition.

Materials and Methods

Cultures of herbivorous zooplankton (*Calanus* sp.), collected from Saanich Inlet waters, were maintained on phytoplankton stocks present in the enclosures, numbered 78-2 and 78-3, which contained predominantly diatom and flagellate populations, respectively. Temperature, salinity, and σ_t data were 15°C, 29.2‰, and 21.5, respectively. Average chlorophyll levels in the "flagellate" and "diatom" waters ranged from 1 to 2 μg/L and cell count data (Utermohl 1958; Strathmann 1967) indicated that flagellates accounted for 96 and 6% of the phytoplankton biomass, respectively. The diatom sample also contained a notable dinoflagellate component, but these organisms did not appear to be grazed appreciably. Culture water was exchanged daily, and sinking rate measurements were done on freshly collected fecal pellets. Pellet size was measured with a calibrated Wild binocular microscope. Pellet volumes (V) were calculated from length (l) and width (w) data by $V = l \cdot \pi \cdot (w/2)^2$, assuming the standard shape of a circular cylinder.

Sinking rate measurements were made via visual observation, timing pellet descent through a nonturbulent water column of given length. Temperature and salinity of the column fluid were 15°C and 29.2‰, respectively. Pellets were allowed to sink untimed for about 7 cm to eliminate momentum due to introduction (Smayda 1969). The distance over which pellets were timed varied between 5 and 33 cm, and analysis of calculated velocities showed no correlation with the path length employed. Fecal pellet sinking rates (ψ) are described by a Stokes-type formulation expressing terminal velocity as the ratio of the forces of gravity and frictional resistance of the media. For a circular cylinder, the gravitational force (F_g) is given by the product of the particle volume, the gravitational acceleration, and the density difference between the particle and the seawater, or

$$(1)\quad F_g = \pi \cdot r_c^2 \cdot l \cdot (\rho_p - \rho_{sw}) \cdot g$$

The frictional resistance (F_R) on a circular cylinder of radius, r_c, is given by Munk and Riley (1952) as

$$(2)\quad F_R = (4 \cdot \pi \cdot \eta \cdot \psi \cdot l)/(2.00 - \ln Re)$$

where η, ψ, l, and Re refer to the medium viscosity, the particle sinking rate, the cylinder length, and the Reynolds number ($Re = 2 \cdot r_c \cdot \rho \cdot \psi/\eta$), respectively; this applies for $Re \ll 1$. No rectification was employed to account for the tapered ends of the pellets. Expressing the cylinder radius in terms of the equivalent radius (r_e) of a sphere of equal volume, and rearrangement of the equation to isolate ($\rho_p - \rho_{sw}$) gives

$$(3)\quad (\rho_p - \rho_{sw}) = (6.604 \cdot \eta \cdot \psi)/[g \cdot q_h^{1/3} \cdot r_e^2 \cdot (2.00 - \ln Re)]$$

where q_h is the cylinder length to radius ratio (l/r_c), and $r_e = r_c(4/3q_h)^{-1/3}$ as shown by Lerman et al. (1974). Estimated values for $\Delta\rho\ (= \rho_p - \rho_{sw})$, given in Table 1, were calculated by conversion of measured hydrophysical, sinking rate and size data to appropriate dimensional units, and substitution to equation 3; pellet density values (ρ_p) resulted from use of $1.0215\ g \cdot cm^{-3}$ for ρ_{sw}.

Results

Analysis of a number of fecal pellets gave the two sets of paired volume-sinking rate data for pellets produced under predominantly diatom- and flagellate-based grazing (Table 1). Pellets produced under the two types of nutrition were roughly similar in size, although pellets from the flagellate case were somewhat larger (Table 1, Fig. 1a). The dimension of fecal pellets produced under a diatom diet showed ranges of length, width, $l:w$ ratio, and volume of 200–500 μm, 40–80 μm, 4.0–12.5, and $0.38–2.51 \cdot 10^6\ \mu m^3$, respectively; under flagellate nutrition pellets had ranges of 200–700 μm, 60–90 μm, 4.0–9.2, and $0.42–3.52 \cdot 10^6\ \mu m^3$, respectively. A plot of fecal pellet sinking rate versus pellet volume is given in Fig. 2 for both sets. There was no significant ($P > 0.05$) correlation between percent sinking rate and volume for either the diatom

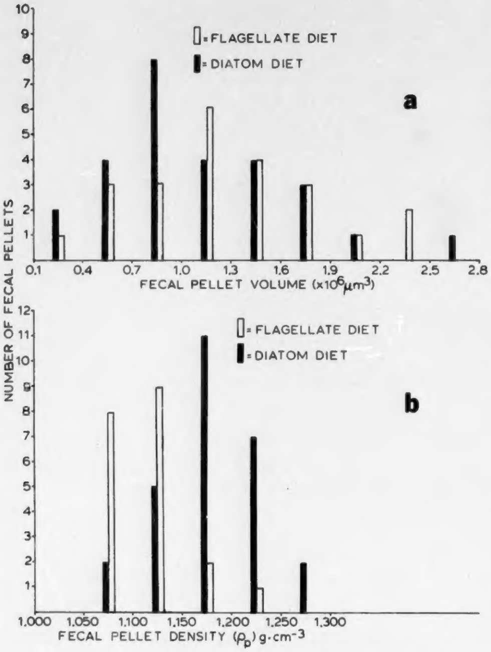


FIG. 1. Frequency distributions of fecal pellet volume (a) and density (b) produced under diatom and flagellate-based nutrition at chlorophyll levels of 1–2 μg/L. Under the flagellate diet $\bar{V}(\pm\mathrm{SE}) = 1.25 \pm 0.11 \times 10^6\ \mu m^3$ and $\bar{\rho}_p$ $(\pm\mathrm{SE}) = 1.114 \pm 0.009 \cdot cm^{-3}$; under the diatom diet $\bar{V}$ $(\pm\mathrm{SE}) = 1.10 \pm 0.09 \times 10^6\ \mu m^3$ and $\bar{\rho}_p$ $(\pm\mathrm{SE}) = 1.174 \pm 0.009\ g \cdot cm^{-3}$.

($r = 0.062$), or flagellate ($r = 0.302$) case, or the two sets together ($r = 0.011$). Neither was there any apparent relationship between sinking rate and pellet size when mean sinking rates were calculated for pellets grouped into several ranges of length and width (Fig. 3a–d).

Average pellet length and width data were 333 × 61 μm and 3.98 × 65 μm for the diatom and flagellate cases, respectively, and in each case the percent standard deviation about the length was greater than that about the width. Despite the similarity in size, the sinking rates of these two sets of fecal pellets were different ($P < 0.01$); average (±SE) sinking rate values ($\psi_d = 123.3 \pm 5.6\ m \cdot d^{-1}$ versus $\psi_f = 87.9 \pm 7.2\ m \cdot d^{-1}$) show fecal pellets produced from zooplankton grazing on a diatom substrate to sink 1.4 × faster than those produced grazing flagellates.

Calculation of net density differences ($\Delta\rho = \rho_p - \rho_{sw}$) gave average values of 0.156 and 0.093 g·cm^{-3} for the diatom and flagellate sets, respectively (Table 1). Average Δ_ρ values for the diatom set were 1.68 × those for the flagellate set. Addition of $\rho_{sw} = 1.0215$ g·cm^{-3} to Δ_ρ terms shows average particle densities (ρ_p) of 1.174 and 1.114 g·cm^{-3} for fecal pellets produced under diatom and flagellate grazing, respectively (Fig. 1b). Using a *t*-test comparison of the means for Δ_ρ and ρ_p between the two data sets show these parameters to be significantly ($P < 0.01$) different under the two types of nutrition. Inspection shows that the measured sinking rate difference (123.3 vs. 87.9 m·d^{-1}) in these two sets of fecal pellets were conferred by particle densities (ρ_p) which differed by only 5.3%. Figure 4 shows a plot of measured fecal pellet sinking rate (m·d^{-1}) against calculated $\Delta\rho$ values in each case; the correlation coefficient for both data sets is $r = 0.85$ and describes significance at $P < 0.01$.

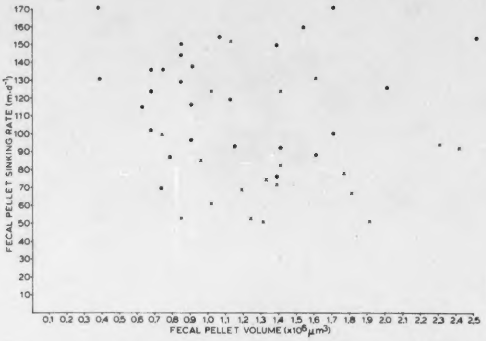


FIG. 2. Distribution of sinking rate (m·d^{-1}) versus volume (10^6 μm^3) of fecal pellets produced by *Calanus* sp. under diatom (●) and flagellate (×) diets. Neither set shows significant correlation at the 5% level.

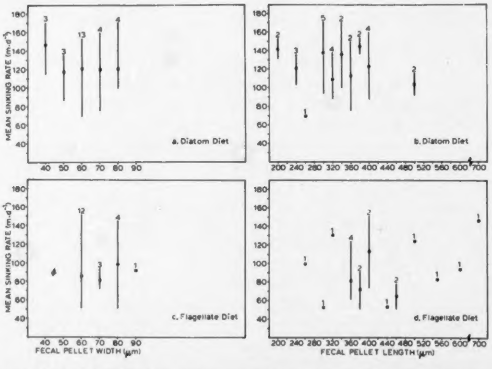


FIG. 3. Distribution of average sinking rates versus length and width parameters of fecal pellets produced under diatom (a, b) and flagellate (c, d) diets (Table 1). The numbers above the range bars give the number of observations involved in each calculation.

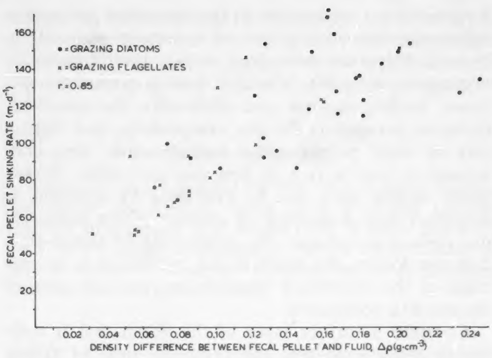


FIG. 4. Sinking rates ($m \cdot d^{-1}$) of fecal pellets produced under diatom (●) and flagellate (×) diets versus the calculated excess density ($\Delta\rho$) of the pellet relative to seawater.

Discussion

The absence of relationship between pellet size and sinking rates in this study differs from the observations made during a number of other studies (e.g. Smayda 1969, 1971; Fowler and Small 1972; Turner 1977; Honjo and Roman 1978). It is clear, however, that in this study, the two groups of fecal pellets with significantly different sinking rates differed otherwise mainly in the diets of the grazers that produced them. Thus, the rate of vertical transport of material by means of pellet sinking can depend on the taxonomic composition of the phytoplankton community. This influence was apparently exerted by resulting changes in the density, rather than size of the fecal pellets observed in this study. Under diatom nutrition fecal pellets averaging $61 \times 333\ \mu m$ sank at $123.3\ m \cdot d^{-1}$, while those produced from flagellate grazing averaged $65 \times 398\ \mu m$ and sank at $87.9\ m \cdot d^{-1}$. The slightly (14%) larger average volume of the latter set did not offset the influence of particle density upon sinking rates. Analysis of the paired data (Table 1, Fig. 2 and 3) showed no apparent correlation between the sinking rate and volume for either set of pellets where pellet volumes ranged from 0.35 to $3.52 \cdot 10^6\ \mu m^3$. The natural variability in fecal pellet sinking rate estimates is quite large as indicated by the 23% standard deviation about the mean of 20 replicate trials of a single pellet (Turner 1977), and the amount of scatter in published sinking rate vs. volume distributions (Smayda 1969, 1971; Fowler and Small 1972; Turner 1977; Small et al. 1979). The comparatively small number of pellets in the two sample sets examined in this study may have contributed to the inability to perceive a relationship between sinking rate and pellet volume recognized by other investigators.

By comparison, small differences (5.3%) in particle density appeared to be more important in influencing ψ_p in pellets produced under the two diets. The calculated average ρ_p under diatom nutrition ($1.174\ g \cdot cm^{-3}$) is similar to the $1.19\ g \cdot cm^{-3}$ value (Dillon 1964) based on pellets collected from the sediment. Compared with these data (Table 1), a $\rho_p = 1.19\ g \cdot cm^{-3}$ value would have given a sinking rate of $130\text{–}140\ m \cdot d^{-1}$, which is only slightly higher than the $123.3\ m \cdot d^{-1}$ value for a diatom diet. Smayda (1969) emphasized caution that fecal pellets collected from the field, whether pelagic or benthic, might become contaminated with sedimentary material. Relatively small amounts of silica or calcium carbonate contamination, for example, could notably affect the sinking rates. The fecal pellets of this study were produced by populations maintained in large glass carboys devoid of sedimentary material, and only freshly collected pellets were inspected. The reported sinking rates therefore do not suffer from the uncertainty due to contamination.

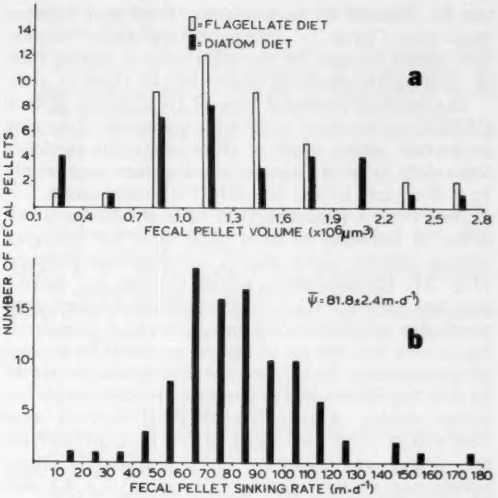


FIG. 5. Frequency distributions for fecal pellet volumes (a) produced under flagellate and diatom diets (chlorophyll levels = 2.5–3.1 $\mu g/L$), when $\bar{V} \pm \text{SE} = 1.29 \pm 0.11 \cdot 10^6\ \mu m^3$ and $1.36 \pm 0.17 \cdot 10^6\ \mu m^3$, respectively. The sinking rate (ψ) distribution of both sets are plotted together in b.

It was not possible to observe pellet orientation during these sinking rate trials. While it is true for inertial systems that falling particles of uniform density will take a preferred orientation with maximum surface area normal to the path of travel, the situation of fecal pellet sinking (Reynolds number $\ll 1$) is a viscous (not inertial) system, and particle orientation can be expected to be random. Variations in orientation might well influence the amount of scatter seen in these sinking rate–volume data (Fig. 2, 3), or by others, e.g. Smayda 1969 (Fig. 1), 1972 (Fig. 2); Fowler and Small 1972 (Fig. 1), Turner 1977 (Fig. 1, 2), and Small et al. 1979 (Fig. 4, 5). Since pellet orientation can be assessed to be randomly distributed between these trials (Table 1), differences in preferred orientation cannot account for the differences in sinking rates of fecal pellets produced under the two types of diets.

The inferred residence time of fecal pellets in both CEPEX enclosures ($z = 20$ m) is quite brief. Assuming an average settled depth of 10 m ($z/2$), the residence times ($10/\bar{\psi}$) in the diatom and flagellate bags would be 0.08 d (1.95 h) and 0.11 d (2.7 h), respectively.

Data from a preliminary set of trials also gave evidence of similarity in fecal pellet size, but divergent sinking velocity under diatom and flagellate nutrition (Fig. 5). Unfortunately, paired volume and sinking rate data were not taken on the individual pellets, thus precluding calculation of ψ and ρ_p values. It is interesting to note that this set of pellets, produced by grazing of considerably higher density phytoplankton stocks (2.5–3.1 μg chlorophyll *a*/L), were generally larger but slower sinking. Average (±SE) pellet volumes were $1.36 \pm 0.17 \times 10^6$ μm^3 and $1.29 \pm 0.11 \times 10^6$ μm^3 for the diatom and flagellate cases, respectively; these pellets had average sinking rates of 83.0 ± 4.1 and 43.6 ± 4.9 $m \cdot d^{-1}$ and potted together (Fig. 5b) these pellets show a generally normal distribution and an overall mean (±SE) sinking rate of 81.8 ± 2.4 $m \cdot d^{-1}$. The suggestion that differences in the concentration (i.e. chlorophyll/L) of the phytoplankton substrate may have been involved in the differences in fecal pellet sinking between this set and those in Table 1 can only be inferred. The different sinking rates of fecal pellets under these flagellate and diatom diets (at similar chlorophyll concentrations) supports the belief that diet can influence the sinking rates of fecal pellets.

That the diet of herbivores will influence the composition of excretory materials produced is axiomatic. The influence of diet upon the sinking rate of zooplankton fecal pellets has been addressed by previous investigations; this influence might result from effects on the compositional density of contained materials, the degree of compaction, and/or the size of pellets produced in different feeding regimes. Marshall and Orr (1955, 1956) stated that the compaction and size of zooplankton fecal pellets varied with diet. Turner (1977) did not find a relationship between pellet sinking rates and the diets of in vitro phytoplankton cultures used to produce the fecal pellets. The composition of fecal pellets produced from grazing upon in vitro phytoplankton diets and those collected from the field is an issue which has been addressed for its relevance to sinking rates (Smayda 1969, 1971; Honjo and Roman 1978; Turner 1977; Small et al. 1979). The results of this study indicate that phyletic differences in the diet can influence the hydrodynamic density of the fecal pellet parcel, and that these density differences can affect the sinking rate of these particles.

The suggestion that characteristics of the primary trophic level may influence the composition, density, and/or sinking rate of excretory products from the secondary trophic level prompted an effort to develop a mathematical description of the functional parameters influencing the vertical flux of excretory materials in the sea. Since the secondary trophic level consists of organisms of various size and having potentially different feeding regimes and efficiencies, the model requires as parameters the size, composition, and sinking rate of fecal pellets under consideration. Any constituent of interest (e.g. carbon, nitrogen, silica, chlorophyll, metals, etc.) can be evaluated by insertion of empirical data describing its content within pellets of the various size-classes. By application of steady-state turnover theory, the result of the calculation is an estimate of the downward transfer rate per unit area of the material considered.

Consider a surface layer of the ocean that is sufficiently shallow so that the residence time of falling pellets is short enough to preclude significant regenerative losses, e.g. remineralization, reingestion. The downward flux of herbivorous fecal pellet material (T_p) can be thought of as the sum of individual loss terms (E_k) for various size-classes (k) of fecal pellets originating from zooplankton of various sizes, where

$$T_p = \sum_{k=1}^{n} E_k.$$

This approach is a convenience for subgrouping and independent analysis of pellet cohorts having similar properties; as a simplification, all pellets are considered below as belonging to one group. The production rate of pellets (P_p) is $P_p = p \cdot B$, where p is the pellet production per organism per unit time, and B is the standing stock of organisms per unit volume producing pellets. If C_p is the steady-state concentration of fecal pellets in the layer, the rate of change in pellet concentration within that mixed layer is given by

$$(4) \quad \frac{dC_p}{dt} = (P_p - T_p)$$

which expresses the balance between pellet inputs due to production (P_p) and losses (T_p) due to sinking. Substitution gives

$$(5) \quad \frac{dC_p}{dt} = [(p \cdot B) - (C_p \cdot \psi_p / z)]$$

where ψ_p is the sinking rate of the pellets and ψ_p/z expresses the pellet fraction which sinks out per unit time. At steady state $dC_p/dt = 0$, and

$$(6) \quad (p \cdot B) = (C_p \cdot \psi / z)$$

which is rearranged to show that the pellet concentration (C_p) at any time is given by the production rate divided by the fraction which has settled out, i.e.

$$(7) \quad C_p = (p \cdot B) \cdot (\psi_p / z)^{-1}$$

From equation 7, the flux of any pellet constituent (x), e.g. carbon, nitrogen, silica, chlorophyll, phaeopigments, etc., is given by

$$(8) \quad (C_p \cdot V_p \cdot k_x \cdot \psi_p) = (z \cdot p \cdot B \cdot V_p \cdot k_x)$$

where V_p is the average pellet volume (cm^3) and k_x is the concentration of parameter x, expressed as weight per unit volume (μg x/cm^3); dimensional analysis of equation 8 gives $x \cdot \mathrm{m}^{-2} \cdot \mathrm{time}^{-1}$, the appropriate units for flux.

Acknowledgments

The work was supported by NSF International Decade for Ocean Exploration Grant OCE 77-27906 for the CEPEX program and NSF Grants OCE 78-19234 and OCE 78-17918. Thanks to the members of the CEPEX staff, to Drs R. Harris, K. King, and C. Davis, and to N. Bienfang for their assistance to this study.

CHERRY, R. D., S. W. FOWLER, T. M. BEASLEY, AND M. HEYRAUD. 1975. Polonium-210: its vertical oceanic transport by zooplankton metabolic activity. Mar. Chem. 3: 103–110.

DILLON, W. P. 1964. Flotation technique for separating fecal pellets and small marine organisms from sand. Limnol. Oceanogr. 9: 601–602.

FERRANTE, F. G., AND J. I. PARKER. 1977. Transport of diatom frustules by copepod fecal pellets to the sediments of Lake Michigan. Limnol. Oceanogr. 22: 92–98.

FOWLER, S. W., AND L. F. SMALL. 1972. Sinking rates of euphausiid fecal pellets. Limnol. Oceanogr. 17: 293–296.

HONJO, S., AND M. R. ROMAN. 1978. Marine copepod fecal pellets: production, preservation and sedimentation. J. Mar. Res. 36: 45–57.

HUTCHINSON, G. E. 1967. A treatise on limnology. Vol. II. Introduction to lake biology and the limnoplankton. J. Wiley, NY. 1115 p.

LERMAN, A., D. LAL, AND M. F. DACEY. 1974. Stokes' settling and chemical reactivity of suspended particles in natural waters, p. 17–47. *In* R. J. Gibbs [ed.] Suspended solids in water symposium proceedings. March 1973. Plenum Press, NY. Mar. Sci. 4.

LOWMAN, F. G., T. P. RICE, AND F. A. RICHARDS. 1971. Accumulation and redistribution of radionuclides by marine organisms, p. 161–199. *In* Radioactivity in the marine environment. Natl. Acad. Sci. Washington.

MARSHALL, S. M., AND P. A. ORR. 1955. On the biology of *Calanus finmarchicus*, VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. J. Mar. Biol. Assoc. U.K. 34: 495–529.

———. 1956. On the biology of *Calanus finmarchicus*, IX. Feeding and digestion in the young stages. J. Mar. Biol. Assoc. U.K. 35: 587–603.

MUNK, H. W., AND G. A. RILEY. 1952. Absorption of nutrients by aquatic plants. J. Mar. Res. 11: 215–240.

OSTERBERG, C., A. CARY, AND H. CURL. 1963. Acceleration of sinking rates of radionuclides in the ocean. Nature 200: 1276–1277.

PORTER, K. G. 1975. Viable gut passage of gelatinous green algae ingested by *Daphnia*. Int. Ver. Theor. Angew. Limnol. Verh. 19: 2840–2850.

SCHRADER, H. J. 1971. Fecal pellets in sedimentation of pelagic diatoms. Science 174: 55–57.

SMALL, L. F., S. W. FOWLER, AND M. Y. UNLU. 1979. Sinking rates of natural copepod fecal pellets. Mar. Biol. 51: 233–241.

SMAYDA, T. J. 1969. Some measurements of the sinking rate of fecal pellets. Limnol. Oceanogr. 14: 621–625.

———. 1971. Normal and accelerated sinking of phytoplankton in the sea. Mar. Geol. 11: 105–122.

STRATHMANN, R. R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411–418.

TURNER, J. T. 1977. Sinking rates of fecal pellets from marine copepod *Pontella meadii*. Mar. Biol. 40: 249–259.

UTERMOHL, H. 1958. Zur Vervollkommung der quantitativen Phytoplankton-Methodik. Int. Ver. Theor. Angew. Limnol. Verh. 9: 1–38.

Reciprocal Averaging and Polar Ordination as Techniques for Analyzing Lotic Macroinvertebrate Communities

JOSEPH M. CULP AND RONALD W. DAVIES

Department of Biology, University of Calgary, Calgary, Alta. T2N 1N4

CULP, J. M., AND R. W. DAVIES. 1980. Reciprocal averaging and polar ordination as techniques for analyzing macroinvertebrate communities. Can. J. Fish. Aquat. Sci. 37: 1358–1364.

Reciprocal averaging and polar ordination techniques were applied to lotic macroinvertebrate field data to determine the relative performance of these techniques in lotic benthic community analyses. It was found that, because of inherent problems with endpoint determinations, polar ordination should only be used where a well-defined environmental gradient exists. Reciprocal averaging ordination produced interpretable axes and was preferred over polar ordination because endpoint determination was objective and simultaneous species–site ordinations were produced. Reciprocal averaging ordination allowed groupings of sites similar to those determined through cluster analysis to be recognized, while providing a visual representation of between-group relationships superior to that of cluster dendrograms. Combined with subsequent analyses of environmental data, reciprocal averaging ordination can be an excellent technique for summarizing spatial and temporal patterns of lotic macroinvertebrate communities.

Key words: ordination, reciprocal averaging, benthic, lotic, communities, clustering

CULP, J. M., AND R. W. DAVIES. 1980. Reciprocal averaging and polar ordination as techniques for analyzing macroinvertebrate communities. Can. J. Fish. Aquat. Sci. 37: 1358–1364.

L'ordination par moyenne réciproque et l'ordination polaire ont été appliquées à des données recueillies sur le terrain sur des macro-invertébrés afin de déterminer la performance relative de ces techniques dans des analyses de communautés benthiques lotiques. À cause de problèmes inhérents aux déterminations des points de virage, la méthode d'ordination polaire ne devrait être utilisée que lorsqu'un gradient bien défini existe dans l'environnement. L'ordination par moyenne réciproque produit des axes interprétables et est considérée préférable à l'ordination polaire parce que la détermination des points de virage est objective et que des ordinations espèces–sites simultanées peuvent être produites. L'ordination par moyenne réciproque permet de reconnaître des groupes de sites semblables à ceux que donne l'analyse de groupes, tout en fournissant une représentation visuelle des relations intergroupes supérieure à celles des dendrogrammes de groupes. Couplée à des analyses subséquentes de données sur l'environnement, l'ordination par moyenne réciproque peut être une excellente méthode pour résumer des schémas spatiaux et temporels de communautés de macro-invertébrés lotiques.

Received November 20, 1979
Accepted May 26, 1980

Reçu le 20 novembre 1979
Accepté le 26 mai 1980

DIRECT and indirect ordination are multivariate statistical methods relating the composition and structure of different communities to one another. Direct ordination arranges samples along one or more ecologically important environmental gradients (Whittaker 1973) and should only be used where the effects of specific environmental gradients are of primary interest (Beals 1973) (e.g. a point source pollutant to a lotic ecosystem). Indirect ordination arranges samples or species along one or more abstract axes which may or may not be related to environmental variables (Whittaker 1973; Austin 1976). Additionally this technique reduces the number of variables to be analyzed by identifying and typifying groups of similar sites and species (Nichols 1977). Thus when spatial and temporal differences in lotic macroinvertebrate community composition are to be studied, indirect ordination techniques are usually more appropriate.

The first effective indirect ordination technique was polar ordination (PO) (Bray and Curtis 1957), and despite several major drawbacks, it is a commonly applied ordination technique in benthic studies (Burlington 1962; Wilhm 1972; Beckett 1977). Because trends in community composition may be obscured by the highly variable observed frequencies of species often obtained in aquatic studies, transformations may be necessary to reduce the weighting of abundant species relative to rare ones. Green (1979) discusses the

Printed in Canada (J5868)
Imprimé au Canada (J5868)

justification of the transformation process and the methods commonly used in environmental studies, while the consequences of centering and standardization on ordination results are presented by Noy-Meir (1973) and Noy-Meir et al. (1975). After appropriate transformation, PO utilizes a dissimilarity matrix usually produced by either percentage similarity (PS) (Czakanowski variant, Bray and Curtis 1957) or Euclidian distance (ED) (Gauch and Whittaker 1972). The relative merits of these and other similarity (or dissimilarity) indices have been extensively discusseed by Williams et al. (1966), Anderson (1971), Gauch and Whittaker (1972), and Clifford and Stephenson (1975), but the determination of the most appropriate index is open to dispute. The major limitation of PO is the subjectivity in the determination of the axis endpoints between which the other samples are ordered. Bray and Curtis (1957) and Beals (1960) described several objective criteria for choosing endpoints, but because of the difficulties experienced when applying these rules (e.g. several different pairs of endpoints each satisfying the objective criteria), Gauch and Whittaker (1972) suggested that PO might be enhanced by eliminating these criteria altogether, and the endpoints should be chosen subjectively.

Two indirect ordination methods free from the significant problems of endpoint choice subjectivity are the eigenvector analyses principal components analysis (PCA) and reciprocal averaging (RA). Since PCA is unsuitable for use with the nonlinear response of species populations to environmental factors (Gauch et al. 1977) it is not an appropriate method for producing axes to be interpreted as environmental gradients, although Nichols (1977) has found it a good technique for data reduction, summarization, and hypothesis generation. Reciprocal averaging (RA) was introduced to ecology by Hill (1973), and Gauch et al. (1977) suggested it should replace PCA because it gave results as easily interpretable as those of PO, and was equally robust. The main advantage of RA over PO and other ordination techniques is the ability to produce simultaneous ordinations for species and sites which are directly related and interpretable (Hill 1973).

As part of a larger lotic benthic macroinvertebrate community analysis (Culp 1978), PO and RA ordination were both applied to a lotic macroinvertebrate field data set to determine (1) the variability in PO results attributable to the choice of different endpoint sets; and (2) the relative usefulness of PO and RA in lotic benthic community analyses. Additionally, classification (cluster) analyses were applied to these field data for comparative purposes.

Reciprocal Averaging

RA ordination is a method of successive approximations which ordinates sites and species on the basis of species distributions (Hill 1973). The process begins by scoring the species from 0 to 100 to approximate their positions along a gradient. Site scores are then produced by averaging the scores of the species which occur at each site. When using quantitative data, this is a weighted average, weights being proportional to the species abundance. Species scores are recalculated from the site scores by averaging the scores of the sites which contain the species. The species scores are rescaled between 0 and 100, and the iteration process continues until site and species scores are stabilized. It is emphasized that the above process produces a one-dimensional ordination which does not depend on the initial weighting of species scores. A second axis is derived by performing the same iteration, except that after each iteration, scores are corrected so that they are uncorrelated with those of the first axis (Hill 1973, appendix II; Gauch 1977).

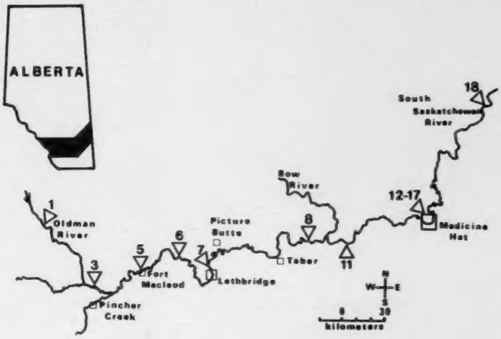


FIG. 1. Location of the sampling sites on the Oldman River (sites 1, 3, 5, 6, 7, and 8) and the South Saskatchewan River (sites 11, 12, 14–18).

Study Area

The study area was located in the South Saskatchewan River Basin, Southern Alberta, with 13 sites ranging along the Oldman and South Saskatchewan rivers (Fig. 1). Based on the physical characteristics of the surrounding terrain, Kellerhals et al. (1972) divided the Oldman River into three zones and placed the South Saskatchewan River into a single relatively uniform zone (Table 1). Five major treated sewage effluents enter the Oldman and South Saskatchewan rivers at Lethbridge, Picture Butte, Taber, and Medicine Hat.

Methods

A common substrate habitat composed of gravel (2–64 mm) and rubble (64–150 mm) at a water depth of 40–60 cm was sampled at all sites. Three replicate samples per site, taken progressively upstream of each other, were collected on each sampling date with a modified Neil (1938) cylinder with 250-μm mesh (Davies et al. 1977). Detritus and invertebrates were separated from the inorganic substrate in the field by swirling the sample in a pail of water, and decanting the suspended organic ma-

TABLE 1. Sites, elevation, mean gradient, and surrounding vegetation of the four zones of the Oldman (zones I, II, and III) and South Saskatchewan rivers (zone IV).

Zone	Sampling sites	Elevation range (m)	Mean gradient ($m \cdot km^{-1}$)	Surrounding vegetation
I	1, 3	1525–1065	5.75	Subalpine forest to *Fescue* Prairie
II	5, 6	945–885	1.33	*Fescue* Prairie
III	7, 8	840–775	1.20	Mixed Prairie
IV	11, 12 14–18	700–610	0.36	Mixed Prairie

terial into a sieve with 125-μm mesh before preservation and storage. Examination of the settled substrate yielded no additional organisms, verifying the effectiveness of the separation technique.

The original monthly data matrices were reduced in size by eliminating all taxa comprising less than 1% of the total number of individuals in a sample. This criterion reduced taxa numbers from 77 to 52 in September, and from 53 to 23 in March. Note that the reduction was meant to eliminate the effects of arbitrarily defined rare taxa which likely were inadequately sampled for quantitative data analysis. A more appropriate method for data screening would have been to employ Orloci's (1973) procedure which ranks the relative importance of species (variables) based on each species' contribution to the total dispersion in the samples.

Two-axis polar ordinations utilizing the percentage similarity coefficient and applying Beals' equations (1960), and two-axis reciprocal averaging ordinations were produced for each month. Classification analyses were calculated using the Euclidian distance measure with Ward's (i.e. minimum variance) agglomerative clustering algorithm (Wishart 1978), and were used to corroborate ordination site-groupings.

Results

Although the study covered an 18-mo sampling period, only the months of September 1975 and March 1976 were selected for comparison of the community analysis techniques. September with high numbers of taxa and sites and March with low numbers of taxa and sites represent the extremes of the range sampled and additionally will demonstrate any sample-size dependence of the analyses.

Three site associations were distinguished in the September RA ordination (Fig. 2a): (a) sites 5 and 6 on the Oldman River; (b) sites 8, 14, and 18; and (c) sites 7, 12, 15, and 16. In addition, the analyses showed the benthic composition of group (b) intermediate between groups (a) and (c). Benthic composition at site 17 varied widely between samples, two samples being included with group (b) and one with (c).

The March RA ordination separated the following site groups (Fig. 2b): (a) sites 5 and 6; (b) sites 12 and 15; and (c) site 14. These groups were similar to those of September, although sites 5 and 6 showed decreased faunal overlap.

To illustrate the variability of polar ordination re-

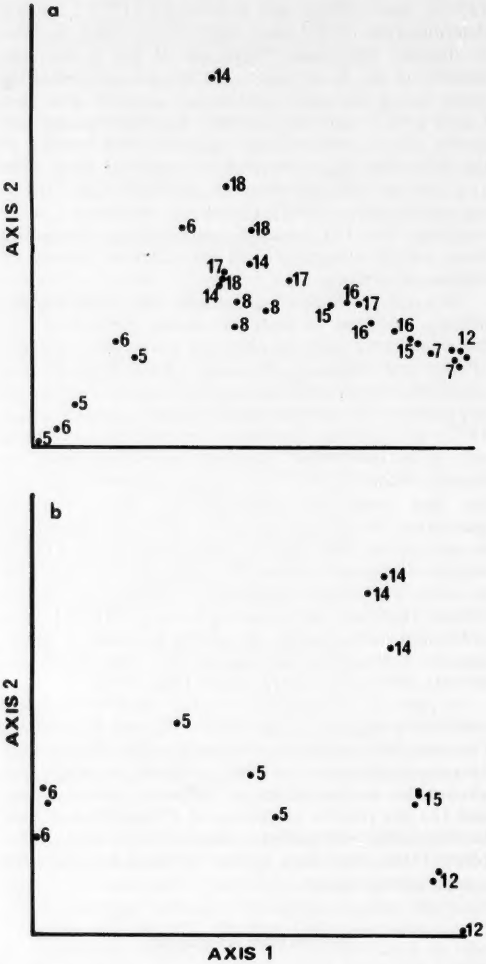


FIG. 2. Reciprocal averaging ordinations for (a) the September 1975 sampling sites; and (b) March 1976 sampling sites. Three replicates per site are plotted.

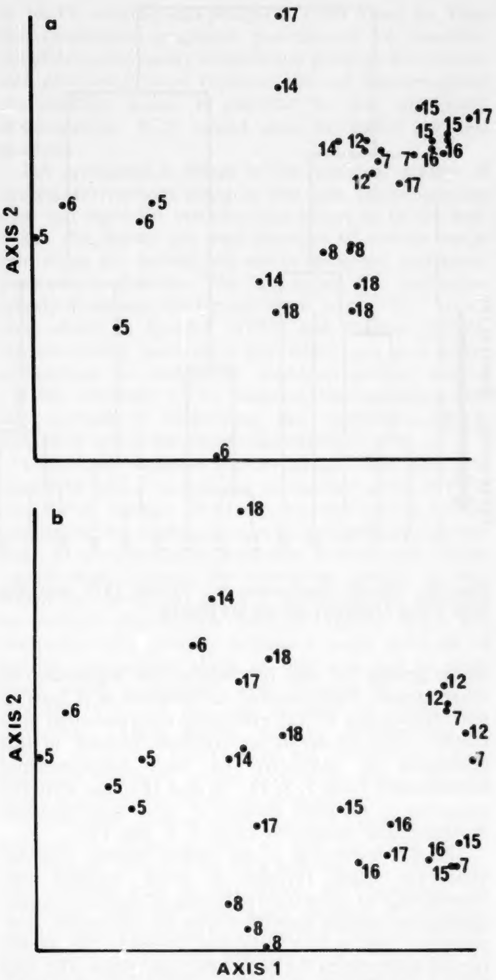


FIG. 3. Polar ordination for (a) September 1975 sampling sites with sites 5 and 17 (abscissa) and sites 6 and 17 (ordinate) as endpoints; and (b) September 1975 sampling sites using sites 5 and 7 (abscissa) and sites 8 and 18 (ordinate) as endpoints. Three replicates per site are plotted.

sults with respect to endpoint choices, two different sets of endpoints, both satisfying the endpoint criteria outlined by Cottam et al. (1973), were selected and used to produce two September site ordinations. Figure 3a utilized site pairs 5 and 17 (abscissa) and 6 and 17 (ordinate), the endpoints of each axis being located near opposite ends of the study area. Compared to the September RA ordination, no major differences were observed, with the exception that site 14 appeared more closely related to sites 7, 12, 15, and 16 in the PO analysis. However, when the PO endpoint pairs were changed to sites 5 and 7 near the center of the study area, and 8 and 18 near opposite ends of the study area (endpoints which also met the objective criteria) (Fig. 3b), the polar ordination differed significantly from both the RA and initial polar ordinations. The formerly tight cluster of sites 7, 12, 15, and 16 appears diffuse, and sites 8 and 18 appear to have highly dissimilar compositions.

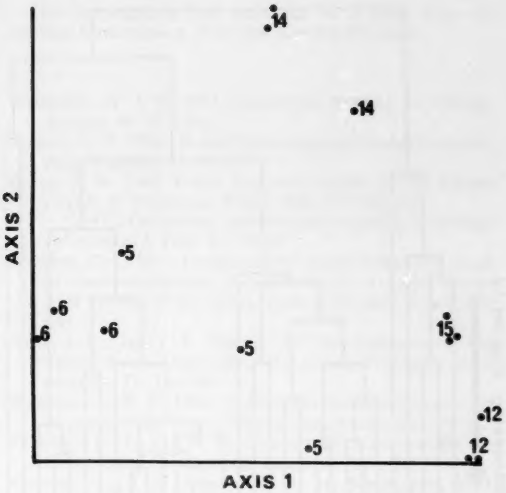


FIG. 4. Polar ordination for the March 1976 sampling sites using sites 6 and 12 (abscissa) and sites 12 and 14 (ordinate) as endpoints; three replicates per site are plotted.

Polar ordination of the March sampling sites (Fig. 4) demonstrated that as the number of endpoint choices decreased, even though different endpoints were utilized by each technique, PO and RA analyses were very similar.

The four-group solution for the September cluster analysis is similar to the RA ordination (Fig. 5a): (a) sites 5 and 6; (b) sites 8, 14, 18, two samples from site 17, and one sample from site 5; (c) sites 7, 12, 15, and 16; and (d) two samples from site 18. These groups correspond with those determined from the RA analysis except that one sample from site 5 is clustered with group (b) above, and two samples from site 18 form a discrete group rather than being associated with group (b) of the RA ordination.

The three-group solution for the March cluster analysis delimited the site-groups (Fig. 5b): (a) sites 5 and 6; (b) site 12; and (c) sites 14 and 15. Although these groups are not identical with those of the RA analysis, they are very similar; furthermore each site's replicate samples showed tight clustering as was the case in the RA analysis.

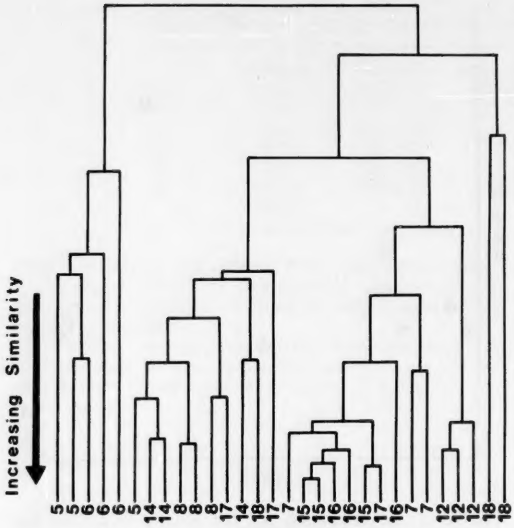


FIG. 5a. Cluster dendogram for September 1975 sampling sites. Three replicates per site are plotted.

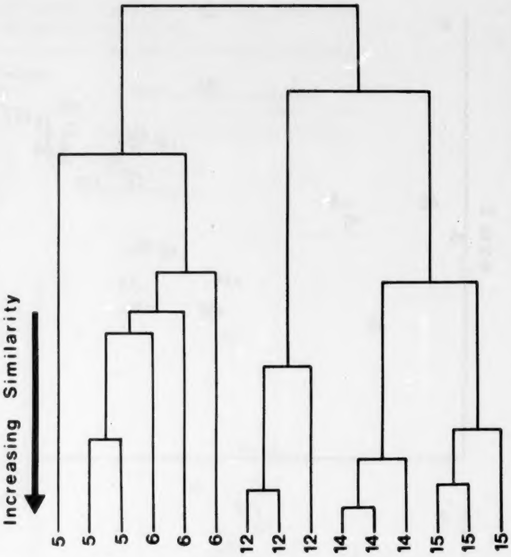


FIG. 5b. Cluster dendogram for March 1976 sampling sites. Three replicates per site are plotted.

Discussion

To utilize successfully ordination techniques for quantitative description of community composition, objectivity is required. As shown for the September samples, the perplexing problem of choosing appropriate endpoints can substantially interfere with successful application of PO. By choosing different endpoint sets, significantly different ordinations are produced. The inherent problems with endpoint choices demonstrate that the use of PO should be strictly restricted to well-defined gradients and endpoints.

Reciprocal averaging ordination (RA) is a superior alternative to PO, since it not only determines endpoints objectively, but also produces a combined species–site ordination. Although RA is mathematically more complex than PO, the techniques produced similar ordinations when RA endpoints were used for PO. Thus the primary defect of PO appears to be the subjective choice of endpoints, not the ordination algorithm.

Results of the RA ordinations for September divided the benthic macroinvertebrate fauna of the Oldman and South Saskatchewan rivers into groups whose boundaries generally coincided with two of the terrestrial botanical zones (Table 1). Sites 5 and 6 (group a) coincide with the Fescue Prairie association, and sites 7, 8, 12, 14, 15, 16, 17, and 18 (groups b and c) to the Mixed Prairie association. Thus it appears that the underlying abiotic factors common to each of these terrestrial zones affects the composition of the benthic communities to some degree, although the variability within groups (b) and (c) indicate the importance of other factors. Since benthic composition is at least in part determined by the prevailing environmental conditions, close clustering of replicate samples in this ordination is indicative of high environmental homogeneity (sites 7, 8, 12, 16, and 18), and scattered clustering of replicate samples of more heterogeneous environmental conditions (sites 5, 6, and 17).

The RA ordination of the March benthic macroinvertebrate fauna revealed a much reduced correspondence to the terrestrial botanical association, suggesting the stresses associated with the low winter temperatures and current velocities overrode the abiotic factors determining the September categories. The high habitat homogeneity during this period, as indicated by the tight clustering of replicate samples at all sites, demonstrates that selective rather than nonselective stresses (sensu Hocutt 1975) produced these responses in the benthic community.

Results of the cluster analyses of the benthic communities produced site-groups which were used to corroborate the RA benthic site-groupings. Cluster dendrograms can be misleading by not clearly representing the relationships between sites or samples which have been delimited to separate groups. For example, the cluster analysis of the September benthic communities classified an outlying sample from site 5 with samples from sites 8, 14, 17, and 18 while the RA ordination clearly shows this outlying sample as being intermediate in composition between group (b) (sites

8, 14, 17, and 18) and group (a) (sites 5 and 6). Thus the continuous graphical presentation of macroinvertebrate community composition given by RA ordination provides a visual representation of between-group relationships which is superior to that of cluster dendrograms. PCA would also be useful for this purpose.

RA ordination is robust to the nonlinear aspects of species distributions along its first axis, but subsequent axes can represent mathematical functions to the first. When this occurs any real direction of sample variation along the second axis can be distorted, and interpretation is dubious. The September RA ordination clearly illustrates this typical curve, termed the "horseshoe effect" by Kendall (1975) and Fasham (1977). An alternative method to RA which can give better ordinations is nonmetric multidimensional scaling (MDS) (Fasham 1977); however, this technique does not succeed in eliminating the "horseshoe effect" caused by strong nonlinear relationships.

Green and Vascotto (1978) suggest that axes produced by linear ordination techniques such as PCA and factor analysis (FA) are not appropriate for investigating the nonlinear responses of species distributions to environmental gradients. Instead they utilize classification analyses for producing groups of sites which may be related to major environmental variables by multiple discriminant analysis (MDA). Since RA ordination will correctly indicate a major direction of species composition along its first axis and in some cases along subsequent axes (Gauch et al. 1977; Fasham 1977), it can also be used to obtain site-groupings. (These groups may be corroborated by cluster analysis.) Additionally RA ordination simultaneously produces species ordination which can aid in the interpretation of the site ordinations.

Community analysis techniques must reduce copious amounts of biological data so that the effect of environmental variables on both community composition and structure may be interpreted. By combining RA ordination with the subsequent analyses of environmental data as outlined by Green and Vascotto (1978), ecologically meaningful analyses of RA axes may be produced. Reciprocal averaging ordination retains much of the information lost by indices which condense the importance values of species into a single numerical value (e.g. species diversity or evenness), and can be utilized to summarize longitudinal and temporal patterns in lotic macroinvertebrate communities (Culp 1978).

Acknowledgments

It is a pleasure to acknowledge the field assistance of G. Scott, M. O'Connell, and Dr Ch. Zimmerman. Thanks are also due to Ms T. M. Elliott for drafting the figures. We would like to thank Dr R. H. Green for his critical analysis which greatly assisted us in the final preparation of this paper and for the courtesy of putting his name on the comments given as referee.

This investigation was supported by a grant from the Alberta Environment, Pollution Control Division.

ANDERSON, A. J. B. 1971. Ordination methods in ecology. Ecology 59: 713–726.

AUSTIN, M. P. 1976. On non-linear response models in ordination. Vegetatio 33: 33–41.

BEALS, E. W. 1960. Forest bird communities in the Apostle Islands of Wisconsin. Wilson Bull. 72: 156–181.

1973. Ordination: mathematical elegance or ecological naivete? J. Ecol. 61: 23–35.

BECKETT, D. C. 1977. Compositional variation and ordination of macroinvertebrate communities in a multi-stressed river system. M.Sc. thesis, Univ. Cincinnati, Cincinnati, OH.

BRAY, J. R., AND J. T. CURTIS. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Monogr. 27: 324–349.

BURLINGTON, R. F. 1962. Quantitative biological assessment of pollution. J. Water Pollut. Contr. Admin. 34: 179–183.

CLIFFORD, H. T., AND W. STEPHENSON. 1975. An introduction to numerical classification. Academic Press, NY. 229 p.

COTTAM, G., F. G. GOFF, AND R. H. WHITTAKER. 1973. Wisconsin comparative ordination. *In* R. H. Whittaker [ed.] Ordination and classification of communities. W. Junk, The Hague. 737 p.

CULP, J. M. 1978. Temporal and longitudinal changes in lotic benthic macro-invertebrate communities. M.Sc. thesis, Univ. Calgary, Calgary, Alta. 166 p.

DAVIES, R. W., J. M. CULP, R. GREEN, M. O'CONNELL, G. SCOTT, AND CH. ZIMMERMAN. 1977. River classification of the South Saskatchewan River Basin. Vol. I. Technical Report, Pollution Control Division, Alberta Environment. 201 p.

FASHAM, M. J. R. 1977. A comparison of nonmetric multidimensional scaling, principal components and reciprocal averaging for the ordination of simulated coenoclines, and coenoplanes. Ecology 58: 551–561.

GAUCH, H. G. 1977. ORDIFLEX — A flexible computer program for four ordination techniques: weighted averages, polar ordination, principal components, and reciprocal averaging. Cornell University, Ithaca, NY. 185 p.

GAUCH, H. G., AND R. H. WHITTAKER. 1972. Comparison of ordination techniques. Ecology 53: 868–875.

GAUCH, H. G., R. H. WHITTAKER, AND T. R. WENTWORTH. 1977. A comparative study of reciprocal averaging and other ordination techniques. J. Ecol. 65: 157–174.

GREEN, R. H. 1979. Sampling design and statistical methods for environmental biologists. Wiley-Interscience, Toronto, Ont. 257 p.

GREEN, R. H., AND G. L. VASCOTTO. 1978. A method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. Water Res. 12: 583–590.

HILL, M. O. 1973. Reciprocal averaging: an eigenvector method for ordination. J. Ecol. 61: 237–249.

HOCUTT, C. H. 1975. Assessment of a stressed macroinvertebrate community. Water Res. Bull. 2: 820–835.

KELLERHALS, R., C. R. NEIL, AND D. I. BRAY. 1972. Hydraulic and geomorphic characteristics of rivers in Alberta. Tech. Rep. 72-1, Alberta Cooperative Research Program in Highway and River Engineering. 156 p.

KENDALL, D. G. 1975. The recovery of structure from fragmentary information. Phil. Trans. R. Soc. London Ser. A, 279: 547–582.

NEIL, R. M. 1938. The food and feeding of the brown trout (*Salmo trutta* L.) in relation to the organic environment. Trans. R. Soc. Edinburgh 59: 383–409.

NICHOLS, S. 1977. On the interpretation of principal components analysis: some problems and developments, Vegetatio 34: 191–197.

NOY-MEIR, I. 1973. Data transformations in ecological ordination. I. Some advantages to non-centering. J. Ecol. 61: 329–341.

NOY-MEIR, I., D. WALKER, AND W. T. WILLIAMS. 1975. Data transformations in ecological ordination. II. On the meaning of data standardization. J. Ecol. 63: 779–800.

ORLOCI, L. 1973. Ranking characters by a dispersion criteria. Nature 244: 371–373.

WHITTAKER, R. H. 1973. Introduction. *In* R. H. Whittaker [ed.] Ordination and classification of communities. W. Junk, The Hague. 737 p.

WILHM, J. 1972. Graphic and mathematical analyses of biotic communities in polluted streams. Annu. Rev. Entomol. 17: 223–252.

WILLIAMS, W. T., J. M. LAMBERT, AND G. N. LANCE. 1966. Multivariate methods in plant ecology V. Similarity analyses and information-analyses. J. Ecol. 54: 427–435.

WISHART, D. 1978. CLUSTAN user manual. 3rd ed. Inter-University/Research Council Series, Rep. 47: 175 p.

Ammonia and Nitrate Uptake in the Lower Great Lakes

T. P. MURPHY

Department of Environment, Aquatic Ecology Division, National Water Research Institute, Burlington, Ont. L7R 4A6

MURPHY, T. P. 1980. Ammonia and nitrate uptake in the Lower Great Lakes. Can. J. Fish. Aquat. Sci. 37: 1365–1372.

Uptake of NH_4^+ is consistent with Michaelis–Menten kinetics in the Lower Great Lakes only if one assumes that the indophenol method of measuring NH_4^+ overestimates the in situ concentration. Short-term incubations were found necessary to avoid induction of nitrate uptake or changes in the rates of NH_4^+ uptake that occurred in long-term incubations. Uptake rates of nitrate are slow most of the summer. Since the nitrate is also present in low concentrations, most of the summer regeneration of nitrate must be very slow. The slow turnover of particulate N, low nutrient concentrations, slow rate of uptake relative to their ability to take up nitrogen, and the high affinity for ammonia (low K_t) suggest that in summer the primary productivity of the Lower Great Lakes is restricted by nitrogen deficiency.

Key words: ammonia, nitrate, Lower Great Lakes

MURPHY, T. P. 1980. Ammonia and nitrate uptake in the Lower Great Lakes. Can. J. Fish. Aquat. Sci. 37: 1365–1372.

L'absorption de NH_4^+ dans les Grands Lacs inférieurs ne s'accorde avec la cinétique de Michaelis–Menten que si l'on suppose que l'indophénol comme méthode de dosage de NH_4^+ surestime la concentration in situ. Il faut des incubations à court terme pour éviter de provoquer l'absorption de nitrate ou les changements de taux d'absorption de NH_4^+ qui se produisent lors d'incubation à long terme. Les taux d'absorption de nitrate sont lents pendant la majeure partie de l'été. Puisque le nitrate est également présent en faibles concentrations, la régénération estivale du nitrate doit être, elle aussi, très lente. Le lent renouvellement de N particulaire, les basses concentrations de nutriments, le lent taux d'absorption par rapport à l'aptitude de ces derniers à absorber l'azote et, enfin, la forte affinité pour l'ammoniac (bas K_t) laissent penser qu'en été, une déficience d'azote limite la productivité primaire des Grands Lacs inférieurs.

Received November 14, 1978
Accepted May 29, 1980

Reçu le 14 novembre 1978
Accepté le 29 mai 1980

THE influence of nitrate and ammonia on lake metabolism has been largely based on indirect measurements: monitor nutrient data and corresponding algal biomass, or algal growth bioassays with lake filtrates. The inadequate quantification of nitrogen reactions in lakes has prevented the direct elucidation of the role of nitrogen in eutrophication. A serious limitation of nitrogen research is the lack of a suitable radioactive tracer and the limited utility of ^{15}N stable isotope techniques.

^{15}N-mass spectroscopy has been used to measure the uptake of nitrogen compounds in natural water samples. This procedure is very time consuming, requires a large sample (up to 500 μg N, Prochazkova et al. 1970), and often necessitates the addition of high concentrations of ^{15}N-labeled nitrogen compounds to the incubation vessel to obtain suitable sensitivity. Unfortunately, the addition of high concentrations of substrate artificially increases the uptake velocity. Many investigators have, consequently, kept the concentration of labeled nutrient to ~10% of the ambient nutrient level. For good analytical sensitivity, this method requires long incubations, 24 h or longer.

^{15}N-NO_3^- and NH_4^+ have been used extensively in marine studies, but only occasionally in freshwater studies to characterize these nutrient fluxes. The uptake of NO_3^- and NH_4^+ has been shown to be linear with time (Dugdale and Goering 1967; Eppley et al. 1969). The uptake of NO_3^- is more light dependent than NH_4^+ uptake (MacIsaac and Dugdale 1972). The kinetics of NH_4^+ and NO_3^- uptake conforms to Michaelis–Menten kinetics in the ocean (Eppley et al. 1969; MacIsaac 1969), in freshwater (Toetz et al. 1973), and in culture (Rhee 1978). However, occasionally Dugdale and Goering (1967) and Toetz et al. (1973) have found that NH_4^+ uptake did not conform to Michaelis–Menten kinetics. This discrepancy has not been resolved.

Since the kinetic method involves the addition of several different concentrations of nutrient, the in situ nitrogen uptake can be calculated by extrapolation (MacIsaac 1969). Most kinetic studies emphasize the uptake constant K_t, the half-saturation constant. K_t is thought to be a measure of the affinity of the uptake system for the substrate (Dugdale 1977). Also, since

Printed in Canada (J5431)
Imprimé au Canada (J5431)

K_t is independent of the amount of detritus (MacIsaac and Dugdale 1972), it should be an excellent index of nitrogen metabolism.

A new, less time-consuming ^{15}N technique, emission spectrophotometry, has been used here to obtain a relatively large data base for nitrate and ammonia uptake kinetics in lakes Ontario and Erie. These lakes have been chosen because several reports (Munawar and Burns 1976; Sridharan and Lee 1977; Lange 1971) have shown by bioassays that nitrogen limits the algal production of these lakes.

Also, the concentration of NH_4^+ and NO_3^- from June to October is insufficient to allow one doubling of the seston (Gachter et al. 1974). Unfortunately, because the uptake or regeneration rates of nitrogen in lakes Ontario or Erie is unknown, this pool size data cannot directly corroborate the bioassay results.

Methods

The Lake Ontario sites were Station A, 43°46′33″N, 78°51′14″W, and Station B, 43°24′10″N, 79°38′50″W. The Lake Erie sites were Station C, 42°18′12″N, 81°11′48″W, Station D, 41°41′06″N, 82°56′00″W, and Station E, 42°35′08″N, 80°00′58″W.

Analysis was started in the laboratory within 2 h. All samples were studied in constant light incubators at the light intensity of the optimal algal activity at Station A, 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. This light intensity was determined by incubating saturating concentrations of NH_4^+, 100 μg N/L, and NO_3^-, 500 μg N/L (separate bottles) at various depths in 300-mL biological oxygen demand (B.O.D.) bottles.

The light response of the algae was also tested in the incubator by attenuating the light with neutral density screens. The shipboard incubator, a Steemann Nielsen design, modified by Fee (1973), used quartz-halogen lamps. The psycrotherm (New Brunswick G-27) incubator, which had cool-white fluorescent lamps, also set at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$, was used with the Station B sample. Light was measured with a quantum sensor (Lambda Instruments). Both types of incubators were able to maintain the in situ temperature to within 1°C.

Linearity of uptake with time was tested several times by incubating 5 m water at both low and high concentra-

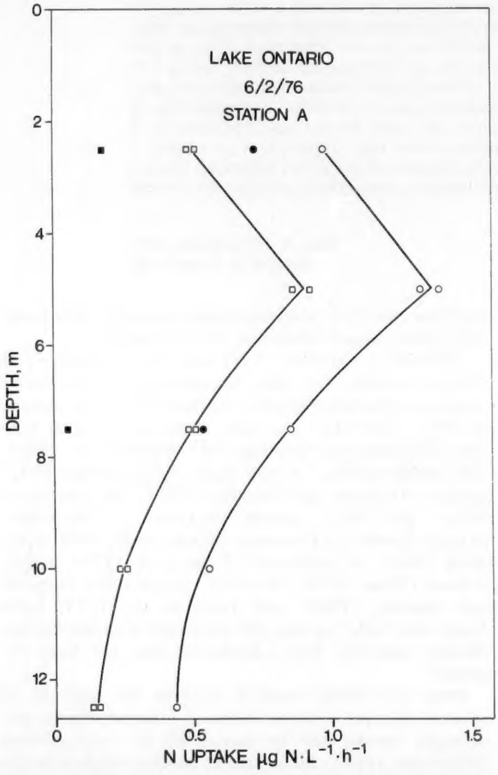


FIG. 1. The in situ uptake of ammonia ○ (100 μg N/L), and nitrate □ (500 μg N/L). The solid symbols ●, ■ are dark incubations.

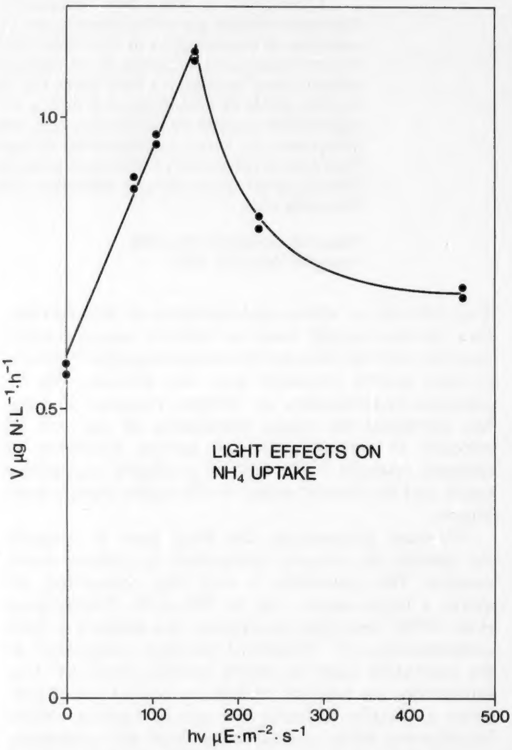


FIG. 2. The light dependency of ammonia uptake (100 μg N/L) in the incubator as measured with a quantum sensor ●.

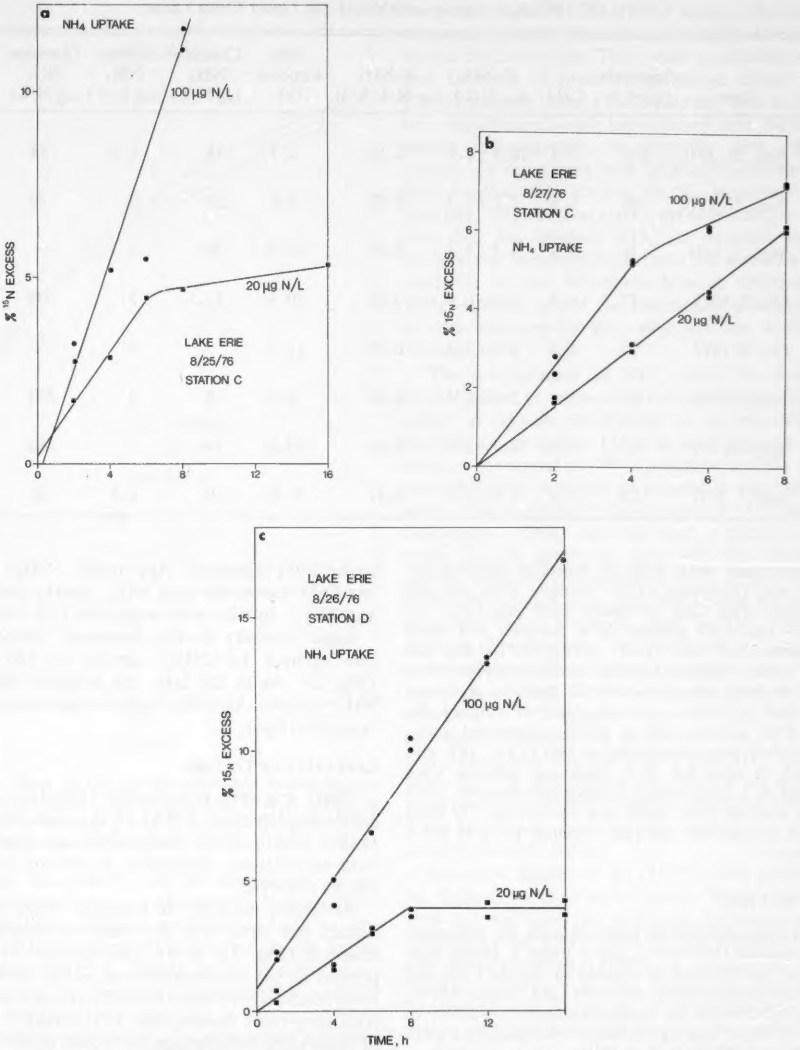


FIG. 3a, b, c. Three long-term incubations in Lake Erie with 20 μg N/L ammonia ■ and 100 μg N/L ammonia ●.

tions of NH_4^+ or NO_3^-. All incubations were done in constant light in the incubator. Each time interval was sampled from a separate B.O.D. bottle.

For kinetic analysis, several B.O.D. bottles of 5 m water were inoculated with different amounts of ^{15}N-NH_4^+ or NO_3^- (97% ^{15}N BioRad). The modified Lineweaver–Burk plot *S*/*V* vs. *S*. (found in any biochemistry text) was used to calculate the in situ uptake and the K_t value.

Uptake of nitrogen was terminated in all experiments by immediate filtration. Samples were washed with 10 mL of 0.2-μm lake water filtrate. The wash water was added to the filtration funnel just before the algal filtration was over. Sample filters were frozen until they could be dried at 50°C and stored in desiccators. Samples for particulate nitrogen and carbon were treated in the same manner. Whatman filters (2.4-cm diam. GF/C) were used for ^{15}N samples and 4.7-cm diam. GF/C filters were used for C/N samples. Filters were preignited at 500°C for several hours before use. Particulate carbon and nitrogen were analyzed on a Carlo-Erba CHN elemental analyzer.

TABLE 1. Nitrogen uptake variables in the Lower Great Lakes.

Lake	Date	Seston-N (μg/L)	C/N	K_t-NH_4 (μg N/L)	V-NH_4 (μg N/L·h^{-1})	NH_4 turnover (h)	Chemical NH_4 (μg N/L)	Kinetic NH_4 (μg N/L)	Chemical NO_3 (μg N/L)	V-NO_3 (μg N/L·h^{-1})
Erie Station D	Aug. 26, 1976	109	5.7	16.8±2.2	2.50	2.7	15	1.5	18	<0.001
Erie Station C	Aug. 27, 1976	96	6.5	5.2±2.2	0.67	5.6	28	9	20	<0.003
Erie Station C	Sept. 7, 1977	78	7.9	4.2±3.1	0.03	10.0	20	5	—	<0.001
Erie Station C	Oct. 5, 1977	97	6.6	3.9±0.9	0.21	10.5	13.5	3	100	0.032
Erie Station E	Oct. 6, 1977	70	6.6	8.0±2.4	0.22	15.0	3	3	—	—
Ontario Station A	June 2, 1976	96	7.4	2.8±1.4	0.40	6.0	8	4	200	0.420
Ontario Station B	May 24, 1977	83	5.8	4.2±2.7	0.05	10.0	10	1	280	0.280
Ontario Station B	June 7, 1977	112	5.8	5.6±1.1	0.11	27.0	10	1.5	20	<0.001

^{15}N measurements were made by emission spectroscopy (Kanazawa and Yoneyama 1976). Samples were put into discharge tubes with CaO to absorb H_2O and CO_2, and with Cuprox and CuO powder as a catalyst. The tubes were evacuated to 10^{-4} torr (1 torr = 101 325/760 Pa) and sealed. For a good vacuum the CaO must be preheated to 1000°C for an hour; the tubes and Cu must be preheated to 590°C; and the tubes must be "excited" during the evacuation with a Tesla coil to dislodge absorbed gases. The sealed tubes were combusted at 590°C for 16 h and then allowed to cool for 24 h. Duplicate samples were then analyzed on a Jasco emission spectrophotometer. Each sample was scanned three times and the average ^{15}N ratio used for the calculation. Samples required 10 μg N for a good emission.

CHEMICAL ANALYSES

Ammonia was analyzed on board ship by the phenolhypochlorite method (Solorzano 1969) using a 10-cm path length. Color development proceeded in the dark to void photochemical enhancement (Gravitz and Gleye 1975). Nitrate was determined by cadmium–copper reduction to nitrite by the Water Quality Section of the Canada Centre for Inland Waters (Philbert et al. 1975).

Results and Discussion

OPTIMAL LIGHT

The in situ incubations (Fig. 1) showed that 5 m was the depth of optimal NO_3^- and NH_4^+ uptake. There was less than a 10% variation in the concentration of NO_3^- (200 μg N/L) or NH_4^+ (15 μg N/L) with depth; therefore, N concentration had no effect on this profile. The uptake of N/μg particulate N had the same depth response; thus, the N uptake appeared to be light-regulated. Apparently, NH_4^+ uptake was less light-dependent than NO_3^- uptake and both NO_3^- and NH_4^+ uptake were suppressed by intense light.

Light intensity in the Steemann Neilsen incubator was optimal for NH_4^+ uptake at 140 μE·m^{-2}·s^{-1} (Fig. 2). As in the lake, the brightest light inhibited NH_4^+ uptake. All NH_4^+ uptake experiments were done in optimal light.

LINEARITY OF UPTAKE

There are very few reports (Dugdale and Goering 1967; Eppley et al. 1969) of the linearity of nitrogen uptake with time for their incubation conditions. With long incubations, significant deviations from linearity can be observed.

Ammonia uptake, in constant light, was initially linear, but after 6 h the rates of ammonia uptake changed (Fig. 3a, b, c). The amount of change depended on the concentration of added ammonia; therefore, Michaelis–Menten kinetic analysis cannot be done with long-term incubations. It is possible that the use of long-term incubations by Toetz et al. (1973) was responsible for the reported irregular ammonia uptake kinetics. These changes in uptake rates could be caused by confinement artifacts, by circadian rhythms, or by feedback control within the algal N uptake system establishing a new equilibrium.

AMMONIA UPTAKE KINETICS

The uptake of NH_4^+, during the present studies, was not directly consistent with Michaelis–Menten kinetics. As the concentration of NH_4^+ increased, the velocity of uptake decreased and then increased. This pattern was the same as that observed by Toetz et al.

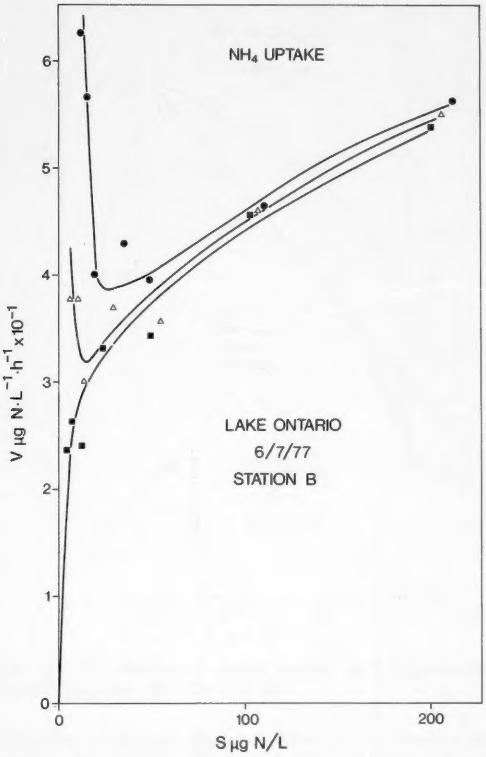


FIG. 4. The effect of the in situ ammonia determination on the ammonia uptake kinetic calculations. ■ is assuming 1.5 µg N/L, △ is assuming 5 µg N/L, and ● is using the chemically determined concentration of 10 µg N/L.

(1973). This irregularity can be eliminated if the chemical determination of ambient NH_4^+ is an overestimate. The term ambient ammonia is used to distinguish the natural concentration of ammonia in the lake from that which is added to an incubation or which is released by chemical degradation of organic matter. By progressively assuming a lower ambient NH_4^+, the uptake curve gradually approaches a typical substrate saturation curve (fig. 4, Rigler 1966). The ^{15}N enrichment values do increase as the added NH_4^+ increases in a manner consistent with Michaelis–Menten theory. The discrepancy arises in the calculation to measure the actual velocity of uptake.

$$V = \frac{\%\ \text{excess}\ ^{15}\text{N in seston} \times \text{particulate N}}{\%\ ^{15}\text{N-NH}_4^+ \times \text{time}}.$$

The $^{15}N\text{-}NH_4^+$ is obviously very dependent on the measurement of the ambient ammonia. A small interference with the ammonia determination would produce poor ^{15}N ratios that would only be apparent in samples where little ^{15}N was added. Previous work by Liao and Lean (1978) found similar disagreement of ammonia kinetics and the chemically determined ammonia concentration. They used an alkaline distillation and titration to measure ammonia. Other studies in prairie pothole lakes (T. P. Murphy and B. B. Brownlee, unpublished data) have shown that, whenever ammonia concentration is naturally high, the uptake curves are consistent with Michaelis–Menten kinetics. Size fractionation studies (B. K. Burnison and T. P. Murphy, unpublished data) provide another confirmation that the ambient NH_4^+ is overestimated. Algae of different sizes separated from the same water sample conform to the Michaelis–Menten interpretation of NH_4^+ uptake only if the chemically determined NH_4^+ is overestimated by the same amount in both calculations.

The overestimate of NH_4^+ may be caused by the hydrolysis of an organic-N compound that decomposes either in alkaline distillations or by reaction with the indophenol reagents. [This is analogous to the problems encountered in ^{32}P kinetics when the concentration of soluble reactive phosphate is used as the available orthophosphate concentration (Rigler 1966).] Solorzano (1969) showed that a mixture of amino acids did not appear to react with the indophenol reagents. However, Brezonik (1973) found that certain important amino acids did react with the indophenol reagents. Other methods such as the rubazoic acid method (Prochazkova 1964) should be tested to find a more realistic NH_4^+ assay for lakes. Also, Liao (unpublished results) found that amino acids did not interfere with NH_4^+ analysis done with a distillation and subsequent titration to determine NH_4^+. Distillations and subsequent ^{15}N analysis of the NH_4^+ immediately after the ^{15}N inoculation could allow accurate calculations of ambient NH_4^+ concentration and NH_4^+ uptake. This technique, however, is tedious, slow, and unsuitable for field work.

Recently, Eppley et al. (1977) have published similar findings in the Pacific Ocean. They concluded that they did not have the sensitivity to measure ammonia accurately. All NH_4^+ uptake data presented here used the Rigler method (1966) to reduce the chemically determined NH_4^+ to that consistent with Michaelis–Menten theory. Summary of the chemically measured NH_4^+ and "kinetic" NH_4^+ can be found in Table 1.

Another discrepancy in the ammonia uptake is the shape of the Lineweaver–Burk plot. Four times in Lake Ontario (Fig. 5a), and once in Lake Erie (Fig. 5b), the S/V vs. S plot consisted of two linear portions, not as would be anticipated. Three midsummer S/V vs. S plots in Lake Erie consisted of one line (Fig. 5c). The discrepancy associated with the ammonia determinations does not affect these observations. If the ammonia value from the indophenol reaction was accepted, the two lines would only be transposed to the right. Each linear portion is probably caused by the uptake of an algal subpopulation with a distinct physiology. The size fractionation study of Burnison

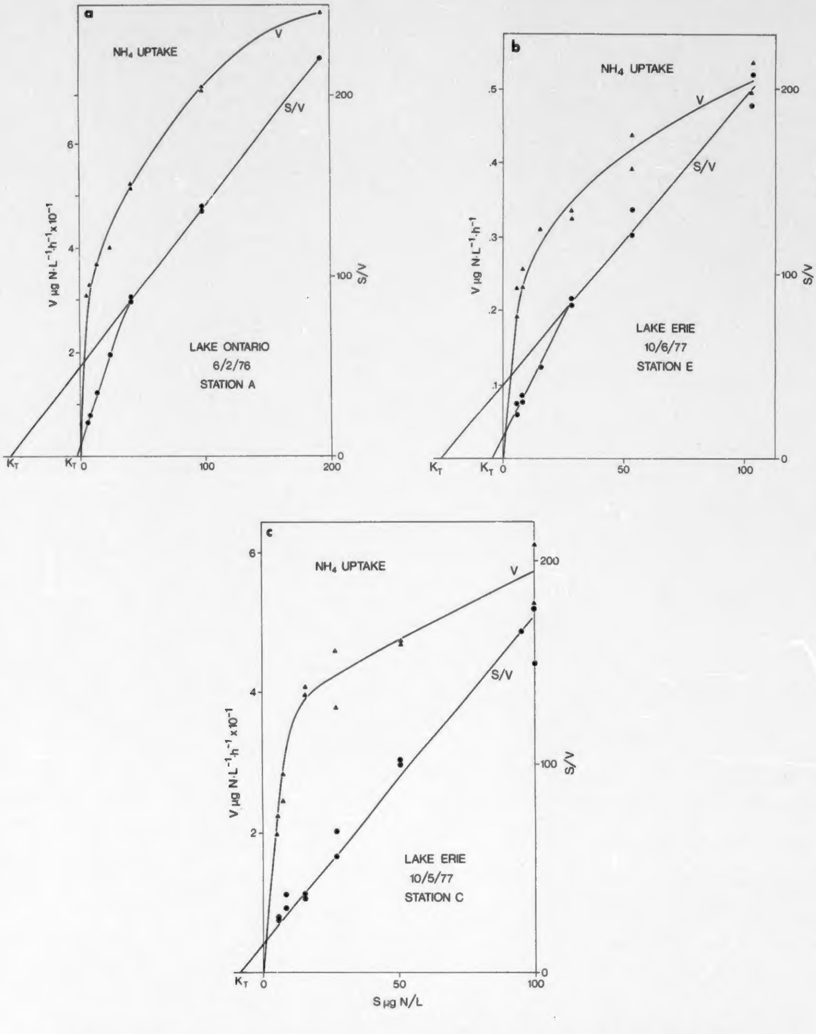


FIG. 5a, b, c. The kinetics of ammonia uptake. ▲ is the velocity of uptake. ● is the S/V plot.

and Murphy (unpublished data) showed that the different size-classes often did have quite different physiologies. In these studies, large diatoms were present only in the spring and fall. The summer algae were much smaller, which is consistent with the view of Eppley et al. (1969) that K_t is related to cell size. MacIsaac (1969) has shown that K_t varies as a function of nitrogen availability. Eppley's studies and MacIsaac's work are complementary. In the ocean, nearshore algae are large, have a high K_t, and are exposed to relatively high concentrations of NH_4^+, while pelagic algae are small, have small K_t values, and are exposed to very little NH_4^+. Eppley and Coatsworth (1968) believed that the algal succession is coincident with a change of K_t. The ability to change its K_t in nature has not been looked for. In culture, K_t varies as a

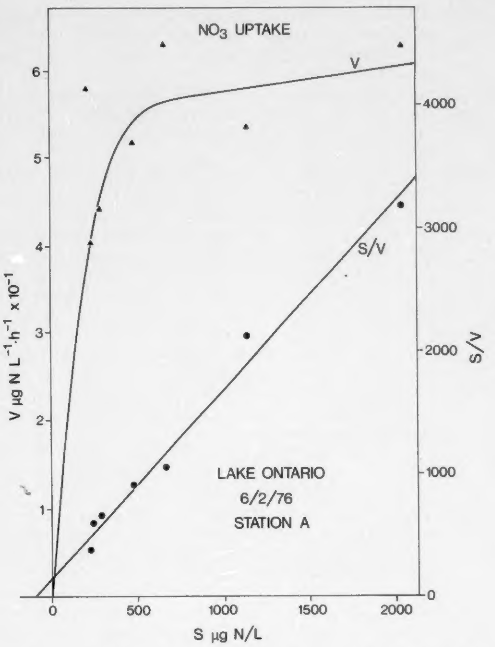


FIG. 6. The kinetics of nitrate uptake. ▲ is the velocity of nitrate uptake. ● is the S/V plot.

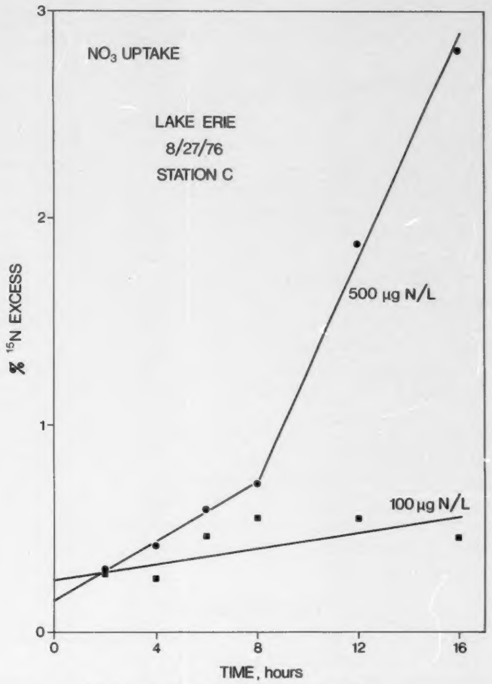


FIG. 7. The inducement of nitrate uptake in long-term incubations. ■ is the incubation with 100 μg N/L. ● is the incubation with 500 μg N/L.

function of nitrogen demand (Rhee 1978). Studies reported here indicate that, with the exception of the sample from the western Basin of Lake Erie, the samples have very similar K_t values. This result may be related to the western basin being much more productive than the other sites studied here (Gachter et al. 1974; Glooschenko et al. 1974).

Another obvious impact of these biphasic results is that MacIsaac and Dugdale's method (1972) of using a Michaelis–Menten transformation to calculate the in situ uptake must be used with a wide range of ammonia concentrations. The use of only high concentrations would allow great analytical sensitivity, but the in situ ammonia uptake could be greatly overestimated.

NITRATE UPTAKE KINETICS

In the Lower Great Lakes, NO_3^- uptake appears to be important for only short periods, in the spring and fall (Table 1). The uptake kinetics in the spring were directly consistent with Michaelis–Menten theory without any adjustments in the ambient NO_3^- value (Fig. 6). However, in the summer, the analytical sensitivity of the ^{15}N is inadequate to measure NO_3^- uptake kinetics with short-term incubations. The ^{15}N enrichments were less than 0.8% excess for high concentrations of NO_3^- (500 μg N/L) and undetectable with low concentrations (<100 μg N/L). Eppley et al. (1969) overcame this problem by preincubating with NO_3^- to induce NO_3^- uptake before doing kinetic uptake experiments. It is uncertain what effect the high concentration of NO_3^- required to induce uptake has on the kinetic constants and the validity of the kinetic interpretation.

A long-term NO_3^- uptake experiment at Station C when NO_3^- was only 17 μg N/L (Fig. 7) showed induction of NO_3^- uptake with 500 μg N/L but not 100 μg N/L NO_3^-. These results suggest that very little NO_3^- is utilized in the summer. Also, since NO_3^- concentrations are low most of the summer (Gachter et al. 1974), nitrification rates must be low in the summer. These results are consistent with marine studies by Dugdale and Goering (1967) and Eppley et al. (1969), but not with freshwater studies by Toetz et al. (1977) and statements by Wetzel (1975). Toetz showed that NO_3^- uptake was maximal in midsummer when NO_3^- concentrations were lowest. The lake he studied may be quite different or his long-term incubations may have induced NO_3^- uptake. Wetzel's statement, low NO_3^- concentration infers high turnover rates, is not apparently based on N studies and probably arises from

V37/9 SE 80 UMI

principles of other kinetic studies such as PO_4 where low concentrations do infer high turnover rates.

Nitrogen Limitation

When the kinetic results obtained here are interpreted using theories derived in the oceans, the Lower Great Lakes appear to be nitrogen limited. The consistent low K_t for NH_4^+ (Table 1) shows that the algae have a high affinity for nitrogen. The limited quantities of NO_3^- and NH_4^+ also suggest that the algae are N-limited. Moreover, this study implies that the NH_4^+ concentration may be overestimated in the present literature. It appears that NO_3^- regeneration is very slow in the epilimnion. Further studies are needed to characterize the regeneration of NH_4^+ and to assess the availability of dissolved organic nitrogen. In spite of the incomplete characterization of all N fluxes, enough has been done to be certain that the algae in both lakes Erie and Ontario would have a greater potential for growth if more nitrogen were available. However, it is premature to assess the amount of control N limitation imposes on primary production relative to other stresses such as P limitation, grazing pressure, light availability, or allelopathic interactions.

Acknowledgments

D. R. S. Lean arranged and organized the Great Lakes cruises. K. Edmondson performed many of the ^{15}N analyses and particulate N and C measurements. C. Nalewajko kindly supplied the Steemann Neilsen incubator. B. K. Burnison and D. R. S. Lean provided valuable criticism of the manuscript.

Brezonik, P. L. 1973. Nitrogen sources and cycling in natural waters. Environ. Prot. Agency 660/3-73-002.

Dugdale, R. C. 1977. Nutrient Cycles. *In* D. H. Cushing and J. J. Walsh. The ecology of the seas. W. B. Saunders Co., Toronto, Ont.

Dugdale, R. C., and J. J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary production. Limnol. Oceanogr. 12: 196–206.

Eppley, R. W., J. H. Sharp, E. H. Renger, M. J. Perry, and W. G. Harrison. 1977. Nitrogen assimilation by phytoplankton and other microorganisms in the surface waters of the Central North Pacific Ocean. Mar. Biol. 39: 111–120.

Eppley, R. W., and J. L. Coatsworth. 1968. Uptake of nitrate and nitrite by *Ditylum brightwellii* — kinetics and mechanisms. J. Phycol. 4: 151–156.

Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14: 912–920.

Fee, E. J. 1973. A numerical model for determining integral primary production and its application to Lake Michigan. J. Fish. Res. Board Can. 30: 1447–1468.

Gachter, R., R. A. Vollenweider, and W. A. Glooschenko. 1974. Seasonal variations of temperature and nutrients in the surface waters of Lakes Ontario and Erie. J. Fish. Res. Board Can. 31: 275–290.

Glooschenko, W. A., J. E. Moore, M. Munawar, and R. A. Vollenweider. 1974. Primary production in Lakes Ontario and Erie: A comparative study. J. Fish. Res. Board Can. 31: 253–263.

Gravitz, N., and L. Gleye. 1975. A photochemical side reaction that interferes with the phenolhypochlorite assay for ammonia. Limnol. Oceanogr. 20: 1015–1017.

Kanazawa, S., and T. Yoneyama. 1976. Rapid determination of ^{15}N-abundance in soil nitrogen by emission spectrographic method. Soil Sci. Plant Nutr. 22: 489–492.

Lange, W. 1971. Limiting nutrient elements in filtered Lake Erie water. Water Res. 5: 1031–1048.

Liao, C. F.-H., and D. R. S. Lean. 1978. Nitrogen dynamics in lake ecosystems: Part II. Transformations within the trophogenic zone. J. Fish. Res. Board Can. 35: 1102–1108.

MacIsaac, J. J. 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep-Sea Res. 16: 45–57.

MacIsaac, J. J., and R. C. Dugdale. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. 19: 209–232.

Munawar, M., and N. M. Burns. 1976. Relationships of phytoplankton biomass with soluble nutrients, primary production, and chlorophyll *a* in Lake Erie. 1970. J. Fish. Res. Board Can. 33: 601–611.

Philbert, F. J., O. Elkei, W. D. Blythe, and Y. M. Sheikh. 1975. Applicability of the technicon autoanalyzer I and II systems for shipboard analysis of Great Lakes water samples. Tech. Bull. 93. IWD, Burlington, Ont.

Prochazkova, L. 1964. Spectrophotometric determination of ammonia as rubazoic acid with bispyrazolone reagent. Anal. Chem. 36: 865–871.

Prochazkova, L., P. Blazko, and M. Kralova. 1970. Chemical changes involving nitrogen metabolism in water and particulate matter during primary production experiments. Limnol. Oceanogr. 15: 797–808.

Rhee, Y. 1978. Effects of N/P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake — a study of dual nutrient limitation. Limnol. Oceanogr. 23: 10–26.

Rigler, F. H. 1966. Radiobiological analysis of inorganic phosphorus in lake water. Int. Ver. Theor. Angew. Limnol. Verh. 16: 465–470.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801.

Sridharan, N., and G. F. Lee. 1977. Algal nutrient limitation in Lake Ontario and tributary waters. Water Res. 11: 849–858.

Toetz, D. W., L. P. Varga, and E. D. Loughran. 1973. Half-saturation constants for uptake of nitrate and ammonia by reservoir plankton. Ecology 54: 903–908.

Toetz, D. W., L. P. Varga, and G. Huss. 1977. Observations on uptake of nitrate and ammonia by reservoir phytoplankton. Arch. Hydrobiol. 79: 182–192.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Toronto, Ont.

Factors Affecting Puget Sound Coho Salmon (*Oncorhynchus kisutch*) Runs[1]

S. B. MATHEWS AND F. W. OLSON[2]

College of Fisheries, University of Washington, Seattle, WA 98195, USA

MATHEWS, S. B., AND F. W. OLSON. 1980. Factors affecting Puget Sound coho salmon (*Oncorhynchus kisutch*) runs. Can. J. Fish. Aquat. Sci. 37: 1373–1378.

Summer streamflow, apparently affecting survival of zero-age coho salmon (*Oncorhynchus kisutch*), is shown to be an important determinant of Puget Sound run strength since 1952. Recently, hatchery production has also been a significant factor. Earlier studies indicated a relationship between rearing flows and coho run strength beginning in 1935. The persistence of the correlation between streamflow and run strength for more than 40 yr is noteworthy. Although the mechanism is unclear, survival of hatchery coho may also be positively dependent upon the same environmental conditions that affect stream-reared coho; a depensatory mortality relationship between abundance of hatchery coho and abundance of stream-reared coho is postulated.

Key words: coho salmon, survival, run prediction, hatchery contribution

MATHEWS, S. B., AND F. W. OLSON. 1980. Factors affecting Puget Sound coho salmon (*Oncorhynchus kisutch*) runs. Can. J. Fish. Aquat. Sci. 37: 1373–1378.

Le débit des cours d'eau en été, qui semble influer sur la survie des saumons coho (*Oncorhynchus kisutch*) d'âge zéro, s'avère un important facteur d'abondance des remontées dans le Puget Sound depuis 1952. Récemment, la production en écloserie a été aussi un facteur important. Des études antérieures ont démontré une relation entre l'écoulement d'élevage et l'abondance des remontées de saumons coho depuis 1935. Cette corrélation entre débit du cours d'eau et abondance de la remontée, qui a persisté pendant plus de 40 ans, est remarquable. Bien que le mécanisme ne soit pas clair, la survie des saumons coho d'écloserie peut également dépendre des mêmes conditions ambiantes que celles affectant les saumons élevés en cours d'eau; nous supposons une relation de mortalité anticompensatoire entre l'abondance des saumons d'écloserie et celle des saumons élevés en cours d'eau.

Received December 12, 1979
Accepted May 27, 1980

Reçu le 12 décembre 1979
Accepté le 27 mai 1980

COHO salmon (*Oncorhynchus kisutch*) is an important species of salmon contributing to commercial and sport catches in Puget Sound. Recent United States commercial net catches in Puget Sound have approached 1 000 000 coho annually, while sport catches have approximated 200 000 (Washington State Department of Fisheries, Annual Statistical Reports). The demand for salmon far exceeds its supply, and this necessitates intense management of the harvest to prevent overexploitation. Successful stock management requires a good understanding of the major factors affecting survival and subsequent run size. This information, in turn, is used in developing run prediction models, which are key tools for managing harvests.

Juvenile coho salmon reside in freshwater through one summer and emigrate to saltwater the following spring as yearling smolts. During their stream residence, young coho are subjected to numerous stresses that diminish the population. These include predation, high temperature, low dissolved oxygen, disease, stranding, and overcrowding which may be associated with streamflow conditions. Smoker (1955) was first to document clearly a relationship between streamflow and Puget Sound coho production. He found a positive correlation ($r = 0.92$, $n = 20$ yr) between the commercial catch of coho salmon from western Washington in year i and the annual water runoff from western Washington streams in year i-2. He also found a lesser but significant correlation ($r = 0.80$) between the June–September runoff in year i-2 and the catch. By about 1967 the correlation between annual runoff and catch had declined, and summer flows were better than annual flows for predicting coho runs.[3] Others (McKernan et al. 1950; Wickett 1951; Neave 1949; Pear-

---

[1] Contribution No. 526, College of Fisheries, University of Washington, Seattle, WA 98195. Supported by U.S. Fish and Wildlife Service by contract No. 14-16-0008-972.

[2] Present address (F.W.O.): CH2M Hill Inc., 1500 114th SE, Bellevue, WA 98004.

Printed in Canada (J5892)
Imprimé au Canada (J5892)

[3] Observation of the senior author when developing a management forecast of the 1968 Puget Sound run for the Washington State Department of Fisheries.

son et al. unpublished MS[4]) had found positive relationships between summer flows and stream survival of coho. For the 1950s and early 1960s a significant positive correlation existed between coho catch by the Oregon commercial troll fishery and total runoff in Oregon coastal streams for the 17 mo each brood spends in freshwater; however, summer flows did not correlate significantly with Oregon catch (D. Scarnecchia, Colorado State Univ., Dep. Fish. Wildl., Fort Collins, CO, personal communication).

During the 1960s and continuing into the 1970s, hatchery production played an increasing role in contributing to net catches of coho salmon. Recent estimates indicate that more than half of all Puget Sound-caught coho are now of hatchery origin (Zillges 1977). Despite the increased hatchery plantings and numerous recent changes in fishing regulations, a relationship persists between summer streamflows and commercial net catches of coho salmon in Puget Sound. The Washington State Department of Fisheries (WDF) continues to depend largely on summer flow data for developing management forecasts of Puget Sound coho runs (Zillges 1977; G. Zillges, Washington State Department of Fisheries, Olympia, WA, personal communication).

The objectives of this paper are to document the continuing relationship between summer flow and run strength of Puget Sound coho, update the run prediction model by regression analysis to account for hatchery production, and indicate that survival of hatchery-reared coho as well as stream-reared coho also may vary with summer flow. The survival dependence of stream-reared coho on summer flows has obvious probable mechanisms. It is less clear why survival of hatchery smolts to adults in year i also should depend on summer flows in year i-2. Possible mechanisms are discussed below.

Methods

ADULT PRODUCTION

Because spawning escapements, other than at hatchery racks and a few fishways, are not enumerated, the best long-term index of coho run strength is the total commercial net catch by American fishermen in Puget Sound (Table 1). This is taken by gill nets, reef nets, and purse seines, mainly in September and October. Prior to 1957 about 25% of the catch was taken by an ocean net fishery in the vicinity of Cape Flattery, but since then ocean net fishing has ceased by United States–Canadian agreement. A portion of the Puget Sound production is harvested in the ocean troll and sport fisheries and is unassignable to Puget Sound. Hatchery mark-recovery studies indicated that the ocean hook-and-line fisheries took about 40 to 50% of the total catch plus escapement for the three consecutive years 1967–69 (Senn 1970a, b; Senn and Satterthwaite 1971). More recent but incomplete mark-recovery data for the 1974–76 runs indicate that the ocean catch fraction may now be more variable than during 1967–69 (Olson 1978), which may be due to changing fishing regulations or hatchery release strategies that affect marine distributions of the fish. A segment of the Puget Sound coho population resides in Puget Sound throughout its marine life. This has been a minor portion of the total (Zillges 1977), except most recently when supplemented by hatchery coho specifically reared and delayed in release date so as to remain inside Puget Sound and contribute to the year-round sport fishery therein (Mathews and Buckley 1976). This "resident" sport catch is not felt to be indicative of overall Puget Sound coho production, and we have not added this to the net catch.

About one-third of the American catch of coho salmon in Puget Sound originates in southern British Columbia, according to interception percentages for various Puget Sound fishing areas generally agreed upon by United States and Canadian scientists for ongoing salmon interception negotiations between the countries (Zillges 1977). It is likely that southern British Columbia and Puget Sound summer steamflows are correlated; therefore it seems valid to attempt prediction of the combined total from Puget Sound streamflow indices.

Catch-per-unit-effort (CPUE) is not considered as good an index of run strength as total catch because both gear and management practices have changed substantially over the years, affecting the coefficient of catchability. However, CPUE correlates fairly well with total catch (Table 1; $r = 0.76$).

STREAMFLOW INDEX

Our index was drawn from U.S. Geological Survey records (U.S. Geological Survey (USGS) 1950–76), which list average daily flow for a number of western Washington streams. We attempted to choose streams that would be geographically representative of the Puget Sound basin, contain coho salmon, and be unaffected by artificial regulation or water withdrawals. The choice was limited to those streams that have had continuously operating gauging stations.

We computed streamflow indices based on the mean daily flows of the 60 lowest consecutive days from June 1 through September 30 for the following Puget Sound streams: South Fork Nooksack River, North Fork Stillaguamish River, Wallace River, Issaquah Creek, Rex River, Newaukum Creek, Greenwater River, Mineral Creek, Deschutes River, and South Fork Skokomish River. The flows for each stream then were converted to percentages of the 26-yr (1952–77) mean. Simple averages of these percentages for the 10 streams were used for an overall Puget Sound index for each year (Table 1). This basic methodology of computing flow indices on the basis of consecutive low flow days was developed by Zillges (1974) for predicting Puget Sound native coho runs returning after 1964.

Summer flows depend upon summer rainfall and snowpack the previous winter. Perhaps widely varying summer flow indices could be developed from the USGS records. However, we found that the choice of streams, time periods, and method of weighting the data are not critical in determining which summers were dry and which were wet.

OTHER VARIABLES

Several other variables were examined by multiple regres-

---

[4] Pearson, L. S., K. R. Conover, and R. E. Sams. Factors affecting the natural rearing of juvenile coho salmon during the summer low flow season. Ore. State Dep. Fish. Wildl. 65 p. mimeo.

TABLE 1. Statistics for prediction of Puget Sound coho net catch.

Year *i*	Puget Sound commercial net catch 000s of fish yr *i*	Puget Sound CPUE[a]	Summer flow index yr *i*-2[b]	Puget Sound hatchery plant t of yearlings yr *i*-1	Washington and Vancouver Island ocean catch 000s of fish yr *i*[d]	Washington troll catch from Westport north 000s of fish yr *i*	Data source
1952	654	17.2	1.19	20.0	1483	768	1. Washington commercial catch and effort 1952–75, Puget Sound, Grays Harbor, Willapa Harbor, and Columbia River bound statistical volumes of the Wash. Dept. Fish. (WDF), Olympia, WA; 1976–78, personal communication, Dale Ward, WDF Olympia, WA.
1953	342	8.1	0.60	20.0	1269	595	
1954	214	6.3	0.72	21.8	901	338	
1955	300	7.0	0.89	36.3	979	412	
1956	409	15.8	1.56	36.3	1372	611	
1957	294	9.9	1.28	25.4	1510	662	
1958	352	10.0	0.93	34.0	1489	523	
1959	348	12.2	0.82	37.2	1577	506	
1960	104	5.2	0.59	30.4	549	131	2. Washington coastal sport catch, 1952–75, 1975 Fisheries Statistical Rep. WDF, Olympia, WA; 1976–77, personal communication, Gene Nye, Olympia, WA.
1961	384	17.4	1.12	34.5	1500	425	
1962	414	16.8	1.08	48.5	1780	524	
1963	233	8.0	0.80	58.5	1730	463	
1964	402	18.0	1.15	35.8	1808	457	
1965	405	17.8	0.94	98.0	2653	650	3. British Columbia troll catches 1952–77, B.C. catch statistics by area and type of gear as reported on sales slips received by the Government. Can. Dept. Fisheries and Oceans, Fisheries Management, Vancouver, B.C. (Title of Dept. as of 1979).
1966	635	26.1	1.82	106.1	2335	701	
1967	288	12.4	0.83	104.8	1955	543	
1968	450	22.6	0.76	203.7	2816	529	
1969	360	19.7	0.65	250.8	1710	306	
1970	850	30.8	1.25	264.0	2249	553	
1971	551	19.1	0.86	311.2	3633	780	
1972	529	19.5	0.77	333.8	1802	346	4. Hatchery plants, 1951–76, annual statistical reports on hatchery production and plantings by the WDF, Olympia, WA; titles of reports are variable
1973	781	19.7	1.20	359.7	2518	593	
1974	875	22.8	1.15	351.5	3037	840	
1975	935	20.2	0.67	424.1	1834	609	
1976	667	14.0	1.09	365.6	3561	1058	
1977	1000	22.0	1.23	426.8	2556	562	
1978	(735)[c]	—	1.47	(407.8)[c]	—	—	

[a] Effort in form of gillnet landings. Purse seine and reef net landings were converted to gillnet units by effort standardization procedures.

[b] Derived as explained in text from flow records for the following streams: Newaukum Creek, Wallace River, N. Fork Stillaguamish River, S. Fork Nooksak River, Issaquah Creek, Rex River, Greenwater River, Mineral Creek, Deschutes River, S. Fork Skokomish River.

[c] Preliminary.

[d] Washington troll and sport catch from Westport north; B.C. troll catch in areas 20–27, and C.

sion analysis to determine their degree of influence, if any, on Puget Sound coho runs. These included (1) streamflows during smolt outmigration (May), (2) estuarial temperatures during smolt outmigration (Elliott Bay), (3) ocean water temperatures (west coast of Vancouver Island) during various periods of the coho's marine residence, (4) growth rates and size during the 1st and 2nd yr of marine residence, (5) ocean troll catches, (6) fishing effort by the ocean troll fishery and by the Puget Sound net fishery, and (7) ocean upwelling indices.

Results and Discussion

Hatchery production has increased very sharply since 1960, increasing catches (Table 1). According to intensive fin-marking studies at all Puget Sound hatcheries for three consecutive brood years, hatchery-reared coho constituted 18% of the 1967–69 Puget Sound net catch (Senn 1970a, b; Senn and Satterthwaite 1971). Release of coho smolts from Puget Sound hatcheries affecting catch during those 3 yr averaged 203 t. Present levels of annual production have risen to 350–400 t, and hatchery fish may now exceed 50% of the catch (Zillges 1977). Hatchery-produced coho, in addition, probably have added to stream spawning escapements, which have been recently showing an upward trend (Washington State Department of Fisheries, unpublished data), and may have tended to increase stream-reared production.

Hatchery coho, which are reared to a yearling stage in the semicontrolled environment of the hatchery, should not be directly affected by summer streamflow conditions. Consequently, the increasing trend in catch of hatchery coho might tend to mask a correlation between summer streamflow and total net catch.

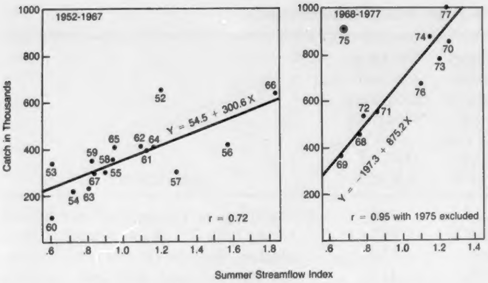


FIG. 1. Puget Sound commercial net catch of coho salmon in year *i* vs. summer streamflow index in year *i*-2.

One way to demonstrate the effect of summer streamflow on catch with the trend in hatchery production removed is to split the data series into an early set, wherein hatchery contribution was relatively minor, and a late set, wherein hatchery contribution was more important. Hatchery production began rising sharply in the mid-1960s; therefore we arbitrarily split the data into a 1952–67 set and a 1968–77 set for separate regressions (Fig. 1). For both sets the relationship between catch and summer streamflow was positive and significant ($P < 0.05$). For the early years $r = 0.72$. For the later set $r = 0.64$; but when the apparent outliner, 1975, is removed, $r = 0.95$. According to mark-recovery data the ocean availability of Puget Sound coho was unusually low in 1975. Of 6 recent years wherein substantial numbers of marked Puget Sound coho were in the fisheries, 1967–69 and 1974–76, 1975 had the lowest ratio of offshore to inshore recoveries (Senn 1970a, b; Senn and Satterthwaite 1971; Olson 1978). Apparently summer streamflow has an important influence on the total coho run size in Puget Sound despite the increased hatchery contribution to the catch.

The trend in hatchery contribution to the catch can also be treated by multiple regression. The following model was assumed:

$$Y = a + b_1X_1 + b_2X_2$$

where,

Y = Puget Sound net catch in year i in thousands of fish.

X_1 = tonnes of hatchery yearling coho released in year i-1.

X_2 = summer flow in year i-2.

This model fitted over the entire 26-yr time period was

$$Y = -25.528 + 1.293X_1 + 316.685X_2$$

In a stepwise regression analysis, hatchery plant was the first independent variable entered ($F = 42.59$, $P < 0.01$) and flow the second (F to enter $= 17.50$, $P < 0.01$). Hatchery plant alone explained 64% of the total variation in catch; and with flow these two independent variables explained 79% of the variation in catch. Serial correlation between hatchery plant (X_1) and streamflows (X_2) was insignificant ($r = -0.01$). The difference between hindcasted catch from this model and actual catch averaged 21% of the actual catch for the 26-yr series.

Inclusion of Puget Sound net fishing effort in the regression improved the model significantly (R^2 from 0.79 to 0.86, $P < 0.01$), but we would exclude this independent variable from a management forecast model because of (1) major gear improvements such as hydraulic drums and deeper and lighter nets from 1952 to 1960 which could not be accounted for in our index of effort, (2) the beginning of intensive terminal-area fisheries in 1973, and (3) parallel trends in fishing effort and hatchery releases since 1960 ($r = 0.79$).

For 1978, final catch and hatchery production values are not available, and one of the streamflow gauging stations used for our index was discontinued in 1976; however, summer flows were above average for all the remaining index streams, and the hatchery plant continued high. The 1978 catch was above average, indicating a good probable fit to the above regression model.

We were unsuccessful in finding any measure of ocean catch or catch-per-unit-effort that was useful for inshore run prediction. There has been a significant positive correlation ($r = 0.60$) between Northern Washington–Vancouver Island ocean catch (Table 1) and Puget Sound net catch but this only reflects the upward trend in both from increased hatchery production. Ocean catch as a third independent variable in attempted stepwise regression analysis did not enter significantly for the entire data series, the early data set (1952–66), or the late set (1967–77). Other measures of ocean abundance attempted in regression included Washington troll catch from Westport north and Washington troll catch-per-landing. The results were inconclusive. For the early data set (1952–67), a good correlation was obtained between Washington troll catch (Table 1) and Puget Sound net catch ($r = 0.81$), but for the entire data series $r = 0.50$, indicating little recent relationship between these two variables.

Other indices of environmental conditions during important life stages of coho salmon (streamflows and estuary temperatures during smolt outmigration, ocean seawater temperatures, fish growth, ocean upwelling during spring of outmigration) did not appear to affect significantly the size of the Puget Sound net catch. The failure of ocean upwelling indices to correlate with Puget Sound catch is noteworthy because Gunsolus[5] (unpublished MS) recently found a high correlation between Oregon coastal upwelling during April–June of year i-1 and Oregon coho salmon production in

---

[5] Gunsolus, R. T. The status of Oregon coho and recommendations for managing the production, harvest and escapement of wild and hatchery-reared stock. Ore. Dep. Fish. Wildl. 59 p. (Mimeo).

TABLE 2. Release size and return rate of Puget Sound hatchery coho.[a]

Year of return i	Avg. wt per yearling released in yr i-1 g (fish/lb)	Avg. return rate (adult return to hatchery in yr i) ÷ (no. yearlings released in yr i-1) × 100	Summer flow index yr i-2
1960	8.8 (51.6)	0.75	0.59
1961	8.1 (56.2)	1.48	1.12
1962	8.5 (53.3)	1.74	1.08
1963	8.8 (51.7)	1.07	0.80
1964	8.0 (56.5)	1.80	1.15
1965	14.4 (31.4)	1.83	0.94
1966	15.8 (28.7)	3.19	1.82
1967	19.7 (23.0)	1.69	0.83
1968	22.0 (20.6)	1.65	0.76
1969	25.9 (17.5)	1.52	0.65
1970	25.9 (17.5)	2.57	1.25
1971	25.8 (17.6)	1.44	0.86
1972	24.8 (18.3)	1.39	0.77
1973	24.4 (18.6)	1.02	1.20
1974	24.8 (18.3)	1.99	1.15
1975	30.5 (14.9)	0.90	0.67
1976	26.9 (16.9)	0.90	1.09
1977	26.2 (17.3)	0.85	1.23

[a] Averaged over the following hatcheries: Nooksack, Samish, Skagit, Skykomish, Issaquah, Green, Puyallup, Minter, George Adams, Hoodsport.

Date sources: Annual hatcheries statistical reports of production and plantings of the Wash. Dept. of Fish., Olympia, 1959–77. (Reports variously titled.)

year i. None of the upwelling indices derived by Gunsolus, including one for the Cape Flattery area, entered into a Puget Sound prediction equation as a significant variable.

There is evidence that adult production of the hatchery-reared segment of the run responds positively to summer flow as does the stream-reared segment. Although the hatchery contribution to the net catch is not precisely known except for years 1967–69 when there were fin-marked coho from all Puget Sound hatcheries, it appears that the relatively good catches from 1973 to 1978 had strong hatchery components. In 5 out of 6 of these years, summer flows 2 yr previously had been above average.

Further evidence of a positive effect of summer flow on hatchery survival is provided by adult return rates to hatchery racks (Table 2). Analyses of such data are complicated by an increasing trend in average release size with time, and by the increasing trend in harvest rate of hatchery coho provided by terminal area fishing. But return rates show good correlation with summer flow indices for return years 1960–77 (Fig. 2). Circled are the points for years 1973–77, the time period for which terminal harvest management policy would have negatively affected hatchery rack return rates. For the remaining years (1960–72) $r = 0.89$, $P < 0.01$. The possibility that nonhatchery fish at the racks may have significantly affected hatchery return rates is unlikely. Most of the hatcheries are on streams so small that nonhatchery abundance at the racks is relatively slight. Washington State Department of Fisheries estimates summarized by Olson (1978) indicate a strong correlation ($r = 0.89$, $P = <0.01$) between hatchery escapement and the total Puget Sound hatchery run (net catches, sport catches, and escapement) for 1965 through 1974. Thus, return rates to the racks prior to changes in terminal area harvest management in the early 1970s are probably good indices of hatchery survival.

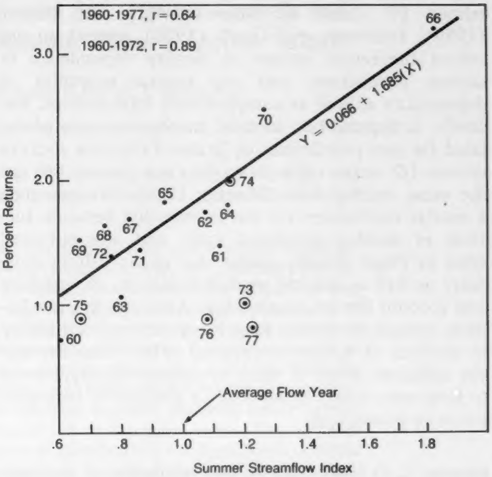


FIG. 2. Adult return rate to Puget Sound hatcheries in year i vs. summer streamflow index year i-2.

Several reasons can be advanced for the positive effect of summer streamflow on hatchery coho survival. First, dry summers are generally warm summers, and hatchery water sources in such summers could become both lower and warmer, stressing the fish without necessarily killing them. This could lead to lower quality smolts. There is little evidence to substantiate this hypothesis. In analyzing rearing records for individual hatcheries, we could find no indication that total rearing mortalities per brood varied with summer flow or that the extent of rearing mortality affected the adult return rate. A more likely hypothesis is that summer flow does not influence hatchery survival directly but that the abundance of nonhatchery fish, varying with summer flow, may affect survival of hatchery fish in the marine environment through depensatory natural or fishing mortality mechanisms. Nonhatchery fish could "buffer" predation or ocean fishing mortality of hatchery fish, causing survival rates of the latter to increase with increasing abundance of the former.

Depensatory mortality mechanisms have been documented for pink salmon (*O. gorbuscha*) and chum salmon (*O. keta*) by Neave (1953) and Hunter (1959). Peterman and Gatto (1978) present an excellent theoretical review of density dependence in salmon populations and cite several examples of depensatory as well as compensatory relationships. Recently, a depensatory survival mechanism was postulated for two populations of British Columbia sockeye salmon (*O. nerka*) that share the same estuary but not the same rearing lake (Manzer 1976). We postulate a similar mechanism for the relationship between survival of hatchery-produced coho and stream-reared coho in Puget Sound, noting that ocean fishing mortality as well as natural predation may be depensatory and account for the relationship. Although this mechanism appears to explain largely year-to-year variability in survival of hatchery-produced coho, there are not yet sufficient years of data to extend this hypothesis to long-term trends in survival as affected by increased hatchery production.

Hunter, J. G. 1959. Survival and production of pink and chum salmon in a coastal stream. J. Fish. Res. Board Can. 16: 835–886.

Manzer, J. I. 1976. Preliminary results of studies on the effects of fertilization of an oligotrophic lake on sockeye salmon (*Oncorhynchus nerka*) in Great Central Lake, B.C. Fish. Res. Board Can. Tech. Rep. 678: 25 p.

Mathews, S. B., and R. Buckley. 1976. Marine mortality of Puget Sound coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 7: 1677–1689.

McKernan, D. L., D. R. Johnson, and J. I. Hodges. 1950. Some factors influencing the trends of salmon populations in Oregon. Trans. N. Am. Wildl. Conf. 15: 437–441.

Neave, F. 1949. Game fish populations of the Cowichan River. Bull. Fish. Res. Board Can. 84: 32 p.

1953. Principles affecting the size of pink and chum salmon populations in British Columbia. J. Fish. Res. Board Can. 9: 450–491.

Olson, F. W. 1978. An evaluation of factors affecting the survival of Puget Sound hatchery-reared coho salmon (*Oncorhynchus kisutch*). M.S. thesis Univ. Washington, Seattle, WA. 72 p.

Peterman, R. M., and M. Gatto. 1978. Estimation of functional responses of predators on juvenile salmon. J. Fish. Res. Board Can. 35: 797–808.

Senn, H. 1970a. Evaluation of 1964 brood coho released from ten Puget Sound and one coastal hatcheries. Wash. Dep. Fish. Prog. Rep. 40 p.

1970b. Evaluation of 1965 brood coho released from ten Puget Sound and two coastal hatcheries. Wash. Dep. Fish. Prog. Rep. 40 p.

Senn, H., and K. Satterthwaite. 1971. Evaluation of 1966 brood coho released from eleven Puget Sound and two coastal hatcheries. Wash. Dep. Fish. Prog. Rep. 42 p.

Smoker, W. A. 1955. Effects of stream flow on silver salmon production in western Washington. Ph.D. dissertation, Univ. Washington, Seattle, WA. 175 p.

U.S. Geological Survey. 1950–76. Surface water resources data for Washington, USGS Tacoma office (reports are variously titled and paged).

Wickett, W. P. 1951. The coho salmon population of Nile creek. Fish. Res. Board Can. Prog. Rep., Pac. Coast Stn. 89: 88–89.

Zillges, G. 1974. 1974 Puget Sound coho run size forecast. Wash. Dep. Fish. Suppl. Prog. Rep. 25 p.

1977. Methodology for determining Puget Sound coho escapement goals, escapement estimates, 1977 preseason run size prediction and in-season run assessment. Wash. Dep. Fish. Tech. Rep. 28. 65 p.

Influence of Parental Smolt Age and Sea Age on Growth and Smolting of Hatchery-Reared Atlantic Salmon (*Salmo salar*)

J. K. BAILEY,[1] R. L. SAUNDERS,[2] AND M. I. BUZETA[1]

Salmon Genetics Research Program, North American Salmon Research Center, St. Andrews, N.B. E0G 2X0

BAILEY, J. K., R. L. SAUNDERS, AND M. I. BUZETA. 1980. Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 37: 1379–1386.

Hatchery-reared Atlantic salmon, *Salmo salar*, consistently developed bimodal length–frequency distributions by the first November following hatch. In a study of 32 hatchery populations involving two year-classes, mean length in the lower mode showed little variation among populations and was not correlated with either the smolt age or sea age at first maturity of their parents. Mean length and the proportion of individuals in the upper mode were both variable among populations and correlated with parental age variables. Large mean length in the upper mode was strongly associated with early smolting male parents and late maturing female parents. The proportion of individuals in the upper mode was more strongly correlated with the age variables of the female parent. Late smolting and early maturing female parents tended to produce relatively greater proportions of fish in the upper mode. The optimum growth rate which produced the greatest proportion of individuals in the upper mode occurred when 8–15% of the total population matured precociously as parr. A threshold size hypothesis for precocious maturation is discussed.

Key words: Atlantic salmon, genetics, growth, bimodality, precocious sexual maturation, smoltification

BAILEY, J. K., R. L. SAUNDERS, AND M. I. BUZETA. 1980. Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 37: 1379–1386.

Les saumons atlantiques (*Salmo salar*), élevés en pisciculture, montrent régulièrement au premier novembre qui suit l'éclosion une distribution bimodale des fréquences de longueur. Dans une étude de 32 populations de pisciculture impliquant deux classes d'âge, la longueur moyenne du mode inférieur accuse peu de variation au sein des populations et ne montre pas de corrélation avec l'âge de smoltification ou le nombre des années en mer avant la première maturité de leurs parents. La longueur moyenne et la portion des individus dans le mode supérieur varient toutes deux au sein des populations et montrent une corrélation avec les variables âge des parents. La plus grande longueur moyenne dans le mode supérieur est étroitement liée à une smoltification hâtive des parent mâles et à une maturation tardive des parents femelles. La proportion des sujets dans le mode supérieur est plus étroitement lieé aux variables âge du parent femelle. Les femelles à smoltification tardive et à maturité hâtive tendent à produire une proportion relativement plus forte de poissons dans le mode supérieur. Le taux de croissance optimal produisant la plus forte proportion d'individus dans le mode supérieur se manifeste quand environ 8 à 15% de la population totale atteignent une maturité précoce comme tacons. Nous analysons l'hypothèse d'un seuil de taille par rapport à une maturation précoce.

Received January 21, 1980
Accepted May 27, 1980

Reçu le 21 janvier 1980
Accepté le 27 mai 1980

HATCHERY-reared Atlantic salmon, *Salmo salar*, families often develop bimodal frequency distributions for length and weight by the first autumn of growth (Thorpe 1977). The two modes contain salmon of both sexes and correspond to subpopulations smolting at t and $t + 1$ yr of age. Thorpe and Morgan (1978) found that 80–90% of the variation among mortality rates and the mean size and proportion of individuals in each mode were influenced by genetic factors. Interfamily variation in the mortality rate and the proportion of individuals in each mode were due to both parents; however, the male parent was more influential. Variation in the mean length of individuals in each mode, on the other hand, was influenced almost totally by the female parent.

---

[1] Huntsman Marine Laboratory, St. Andrews, N.B., E0G 2X0.

[2] Department of Fisheries & Oceans, Biological Station, St. Andrews, N.B.

Printed in Canada (J5939)
Imprimé au Canada (J5939)

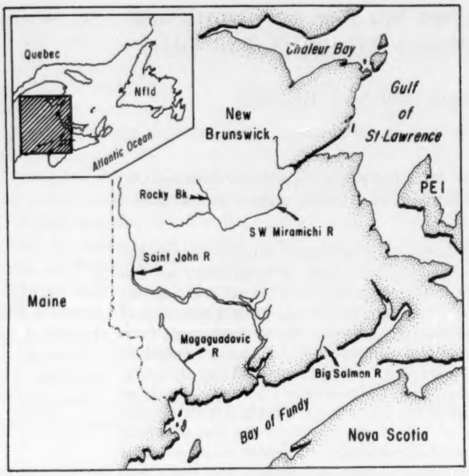


FIG. 1. Locations of the rivers from which wild Atlantic salmon broodstock were collected.

Bimodal patterns of growth and their relationship to age at smolting have important economic implications for the controlled production of Atlantic salmon. Hatchery facilities are used most efficiently in the production of 1-yr smolts and the predictability and manipulation of genetic and environmental influences affecting the proportion of individuals and the growth rate in each size mode are of considerable importance. This paper reports on the occurrence of bimodal frequency distributions for length in hatchery-reared, underyearling Atlantic salmon in New Brunswick, Canada. We examined the effects of parental smolt age and sea age at first maturity on the size modes of 32 hatchery populations in two year-classes.

Materials and Methods

Wild Atlantic salmon broodstock were collected in 1975 and 1976 from four New Brunswick rivers, the Magaguadavic, Saint John, Big Salmon, and Rocky Brook (Fig. 1). Gametes from these fish were combined in a number of 4 × 4 diallel mating sets, producing a total of 16 different strains. Details of the mating design and initial rearing procedures are described by Friars et al. (1979). Eleven and 12 complete sets were produced in 1975 and 1976, respectively. All spawning was done in late autumn and each cross (full sib family) was reared in an individual tank until late June. The parr (average weight 3–4 g) were then counted by family and combined by strain in outdoor rearing tanks (8 m diam.) where they remained until release the following spring. A practical technique for batch marking each cross was not available and family identity was lost at this time.

Parr lengths of the 1975 year-class were determined (nearest 1 mm) in monthly samples of ~100 fish from each strain from July to November 1976. Sampling of the 1976 year-class was done only in November 1977 and immediately before smolt release in May 1978. Subsequent comparisons of length–frequency distributions between year-classes were made on the fish sampled in November. The Cassie (1954) method was used to separate modes and estimate the mean length of each mode of all bimodal distributions. The proportions of fish in each mode, clearly defined in May 1978, were compared to those of the previous November to determine if the modes were sufficiently well defined in November to use this method.

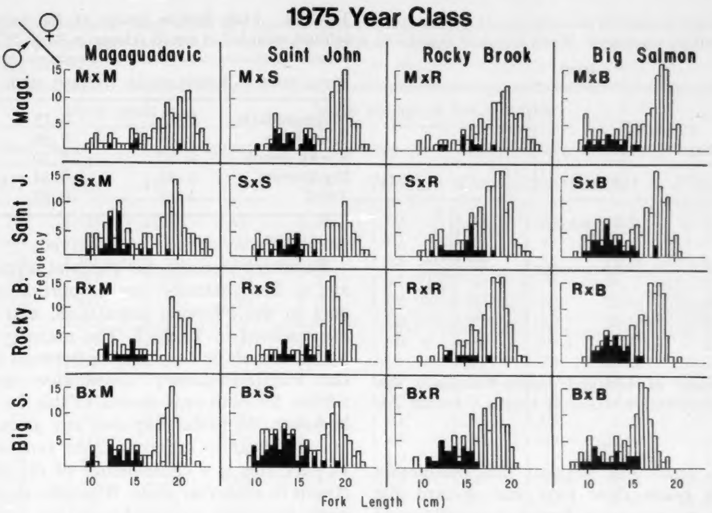
Parental ages were determined by scale reading. Age at smolting and sea age at first maturity were weighted and averaged for each strain. The ages of parents producing larger numbers of surviving parr were given proportionately greater weight in the calculation of means. The issue of milt caused by gentle abdominal pressure identified precociously mature male parr in the November samples. The May 1978 samples of the four pure strain populations were killed to estimate the sex ratios in each mode.

The mean fork length of the lower and upper modes and the proportion of individuals in the upper mode were determined for each sample length–frequency distribution. The strain effect on the variation within each of these parameters was examined by analysis of variance. The influence of parental smolt age, sea age at first maturity, and the proportion of precociously mature males in the offspring populations on the same parameters were examined by partial correlation methods (Snedecor and Cochran 1967). Partial correlations were used to remove any environmental effects caused by differences in rearing densities. Analyses were made on each year-class separately and on both year-classes combined. The probability that each partial correlation coefficient did not differ from zero was determined by calculating the t statistic for each coefficient and evaluating the t distribution density function (Sokal and Rohlf 1969). The angular transformation ($F(x) = \sin^{-1}\sqrt{x}$) was used to stabilize the variance of all percentage data.

Results and Discussion

Length–frequency distributions were clearly bimodal in most strains by the first November following hatch (Fig. 2). Separation into distinct size modes was rapid and could be traced through the monthly sampling of the 1975 year-class (Fig. 3). In July, all distributions were unimodal and slightly skewed, most frequently to the left. As the population aged, the distributions became platykurtic and skewing was more pronounced. By September, two modes could be seen in many strains. During the period from November 1977 to May 1978 there was an increase in mean lengths of the fish in both modal groups. However, the increase in mean length of the upper size mode was greater, indicating relatively faster growth in that mode. At smolt release in May 1978, separation of the 1976 year-class was well defined with little or no overlap between modes (Fig. 4).

The Cassie method of separating modes in polymodal distributions is most reliable when there is little overlap between modes. When the proportions of fish in each mode in May 1978 were compared with those of the previous November, a significant difference


1975 Year Class

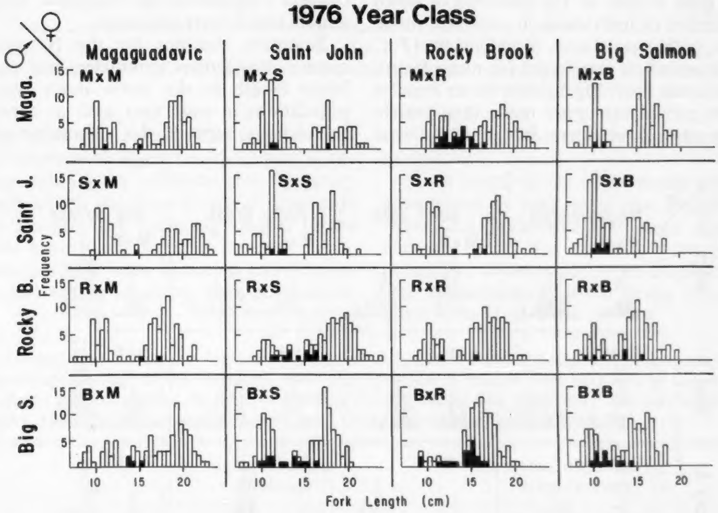

1976 Year Class

FIG. 2. Length–frequency distributions of hatchery-reared Atlantic salmon produced in two diallel experiments and measured during the first November after hatch. Solid bars correspond to precociously mature male parr.

($\chi^2 = 11.3$) was found for only one of the 16 comparisons. This may have been caused by sampling error and it was concluded that modal separation in November was sufficiently well defined to use this technique.

Males and females were found in both modes in the four pure strain populations examined during May 1978 (Fig. 4). The modal trends were consistent in all strains except Rocky Brook, which had a small proportion of individuals in the lower mode and a large number of males in the total sample. In total, the lower size modes had more males and the upper size modes contained more females (Table 1). Although our

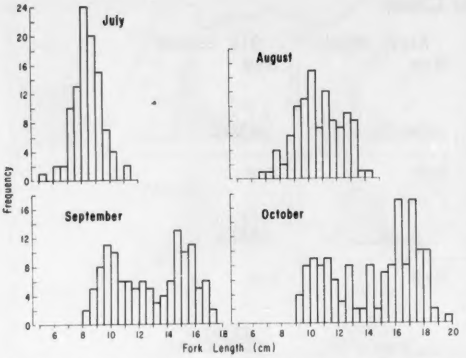


FIG. 3. Development of bimodal length–frequency distribution in a representative strain of hatchery-reared Atlantic salmon.

sample was very small, this suggests that production hatcheries which grade their parr and discard the smaller fish may be removing higher proportions of males than females.

The effect of river strains on the variation in mean length and proportion of individuals in each size mode was minimal. In both year-classes a significant ($P < 0.05$) maternal strain effect was found for mean length in the upper size mode only. Big Salmon River females produced smaller parr in the upper mode than female parents from the other three rivers. Effects of paternal strain differences were not observed.

TABLE 1. Male/female ratios of hatchery-reared Atlantic salmon sampled at smolt release in May 1978.

River strain	Lower mode	Upper mode	Total sample
Magaguadavic	0.92	0.75	0.82
Saint John	1.44	0.69	0.96
Rocky Brook	0.60	1.70	1.44
Big Salmon	1.44	0.64	0.85
Total	1.30	0.87	0.99

Summary statistics for parental mean smolt age, sea age at first maturity, the proportion of mature male parr in the offspring population, and rearing density are outlined in Table 2. The majority of comparable partial correlation coefficients between these values and the length–frequency distribution parameters were similar for both year-classes (Table 3). In many cases, however, the probability that any given coefficient was not significantly different from zero was quite large. In part, this is a consequence of the small number of strains in each year-class. When the degrees of freedom were increased by combining the values from each year-class, the significance of many of the partial correlation coefficients was increased. This indicates that annual trends were consistent.

Summary statistics for the November length–frequency distribution parameters are given in Table 4. Mean length in the lower mode was similar in all populations in each year and, as a result, the partial correlations between this parameter and each of the

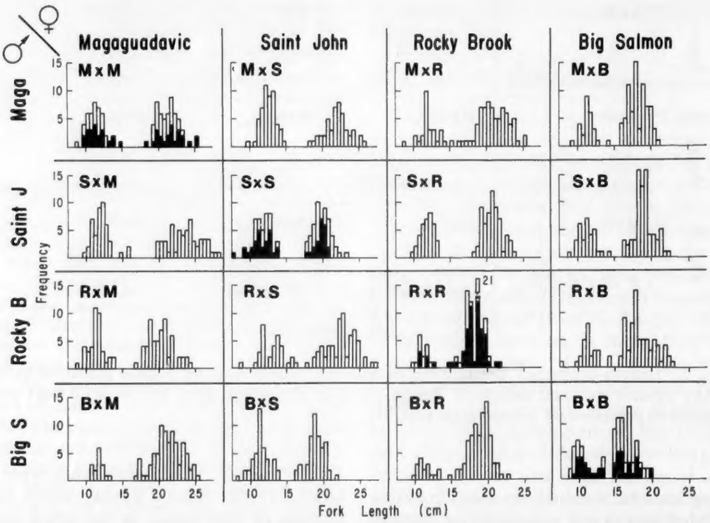


FIG. 4. Length–frequency distributions of the 1976 year-class of Atlantic salmon measured at smolt release in May 1978. Solid bars in the pure strain crosses (leading diagonal) represent males.

TABLE 2. Summary of parental mean smolt age and sea age at first maturity, the proportion of mature male parr progeny, and rearing tank density of hatchery-reared Atlantic salmon which developed bimodal length–frequency distributions by the first November following hatch.

	Mean smolt age				Mean sea age at first maturity				% mature males in progeny		Tank density	
	Male parent		Female parent		Male parent		Female parent					
Genotype[a]	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
MM	2.24	2.00	2.28	2.00	1.56	1.00	2.00	2.00	5.0	2.0	712	970
MS	2.48	2.00	2.31	2.07	1.72	1.02	2.00	1.98	20.0	3.0	984	1132
MR	2.15	2.00	2.76	2.00	1.71	1.15	1.66	1.94	9.0	19.0	1205	1133
MB	2.48	2.00	2.20	2.00	1.77	1.11	1.62	1.78	14.0	2.0	1428	2314
SM	2.22	2.60	2.30	2.00	2.00	2.00	2.00	2.00	20.0	3.0	552	341
SS	2.34	2.85	2.43	2.08	2.00	2.00	2.00	1.89	21.0	3.0	1022	2499
SR	2.32	2.95	2.60	2.00	2.00	2.00	2.00	2.00	14.0	3.0	1200	1014
SB	2.13	2.81	2.31	2.00	2.00	2.01	1.61	1.74	23.0	6.0	1281	2381
RM	2.36	3.00	2.24	2.00	1.48	1.77	2.00	2.00	18.0	2.0	445	757
RS	2.64	3.00	2.30	2.00	1.33	1.75	2.00	1.92	14.0	16.0	854	385
RR	2.53	2.84	2.63	2.00	1.53	1.41	1.69	2.00	8.0	2.0	901	849
RB	2.78	3.00	2.22	2.00	1.19	1.45	1.70	1.69	22.0	7.0	929	1873
BM	2.44	2.60	2.28	2.33	1.70	1.09	2.00	1.84	22.0	4.0	600	533
BS	2.74	2.40	2.38	2.68	1.44	1.24	2.00	1.81	38.0	14.0	729	1663
BR	2.24	2.90	2.58	2.10	1.83	1.26	1.83	2.00	26.0	22.0	1211	1466
BB	2.86	2.60	2.23	2.00	1.27	1.54	1.67	1.80	19.0	12.0	1164	2766
Mean	2.43	2.60	2.38	2.08	1.66	1.49	1.85	1.90	18.3	7.5	995	1380
SD	0.23	0.39	0.17	0.18	0.27	0.38	0.17	0.11	7.9	6.8	287	786

[a] The letters identifying the genotype correspond to the first letters of the rivers from which the broodstock were collected. The first letter identifies the male parent and the second letter identifies the female parent. e.g. BS = Big salmon male × Saint John female.

parental age variables were weak. The absence of a strong parental influence on mean length of parr in the lower mode may indicate the influence of a regulatory mechanism which affects the growth rate of those parr which will not smoltify the following spring. If the purpose of such growth regulation is to concentrate parr of the lower mode at some optimum size prior to their second winter before smolting, then parental influences on growth may be repressed in a November sample.

Mean length in the upper mode was variable among populations in both years and partial correlations between this parameter and mean parental smolt age and sea age at first maturity were generally stronger than those observed for mean length in the lower mode. The relationship between mean length in the upper

TABLE 3. Partial correlation coefficients between parameters determined from bimodal length–frequency distributions and parental mean smolt age, sea age at first maturity, and the proportion of mature male parr among the progeny in hatchery-reared Atlantic salmon sampled during the first November following hatch. The probability that coefficient is equal to zero is given in parentheses. The effect of variation in rearing tank density is removed in each coefficient.

		Parental mean smolt age		Parental mean sea age at first maturity		
Distribution parameter	Year-class	Male	Female	Male	Female	% mature males among progeny
Mean length	1975	−0.18 (0.31)	0.18 (0.31)	0.11 (0.36)	0.53 (0.04)	0.04 (0.39)
Lower mode	1976	−0.12 (0.35)	0.47 (0.07)	0.35 (0.16)	0.25 (0.25)	−0.36 (0.15)
	Comb.	−0.27 (0.13)	0.30 (0.10)	0.37 (0.04)	0.02 (0.39)	0.29 (0.11)
Mean length	1975	−0.67 (0.01)	0.35 (0.16)	0.38 (0.13)	0.64 (0.01)	−0.08 (0.37)
Upper mode	1976	−0.60 (0.02)	0.00 (0.99)	0.31 (0.19)	0.37 (0.14)	−0.33 (0.17)
	Comb.	−0.61 (0.00)	0.26 (0.14)	0.27 (0.13)	0.39 (0.03)	−0.02 (0.39)
Proportion in	1975	−0.09 (0.37)	0.30 (0.20)	−0.20 (0.29)	−0.36 (0.15)	−0.82 (0.01)
upper mode	1976	0.38 (0.13)	0.13 (0.35)	−0.22 (0.28)	−0.13 (0.35)	0.49 (0.06)
	Comb.	0.14 (0.29)	0.33 (0.07)	−0.10 (0.34)	−0.36 (0.05)	0.04 (0.39)

TABLE 4. Summary of mean lengths of parr in the lower and upper modes and the proportion of fish in the upper mode determined from bimodal length–frequency distributions of hatchery-reared Atlantic salmon sampled during the first November following hatch.

	Mean length lower mode		Mean length upper mode		Percent in upper mode	
Genotype	1975	1976	1975	1976	1975	1976
MM	12.6	10.6	19.4	18.7	76	60
MS	13.6	10.9	19.3	18.5	68	40
MR	11.1	11.7	18.3	17.9	89	60
MB	11.2	9.6	17.2	16.2	70	67
SM	12.2	11.0	19.2	19.8	61	62
SS	12.4	12.0	19.1	17.0	61	52
SR	11.3	12.0	17.2	17.8	85	60
SB	11.6	9.7	17.3	15.2	65	40
RM	13.2	9.7	19.5	17.2	65	70
RS	12.4	10.8	18.2	18.0	79	80
RR	13.6	10.0	18.3	16.8	73	84
RB	11.8	9.5	16.6	15.2	72	84
BM	12.8	10.3	18.8	18.0	67	72
BS	12.2	8.0	18.6	16.7	45	68
BR	12.8	8.0	17.4	16.0	70	89
BB	10.5	9.6	15.5	14.9	78	67
Mean	12.2	10.2	18.1	17.1	70.3	65.9
SD	0.91	1.21	1.15	1.39	10.42	14.38

mode and the parental mean smolt age was much stronger for the male parent than the female parent. In addition, paternal and maternal influences exerted opposing effects. Larger mean length in the upper mode was strongly favored by early smolting male parents and, to a lesser extent, by late smolting female parents. Partial correlations between mean length in the upper mode and parental sea age at first maturity were positive for both parents and the maternal values were somewhat greater. Greater mean length in the upper mode was associated with parents that spend relatively longer periods at sea before maturing. These results indicate that mean length in the upper mode may be related to age at various life stages of both male and female parents.

It has been hypothesized that a critical minimum length must be attained in autumn before the "decision" to smoltify the following spring can occur (Elson 1957). Ritter (1975) gives evidence that 2+ hatchery-reared smolts tend to produce greater numbers of grilse (one sea winter) whereas 1+ smolts are more likely to mature after two sea winters. This agrees with the observed paternal partial correlations with mean length in the upper mode and suggests that paternal growth rate in freshwater may exert a strong genetic influence on the pre-smolt growth of their progeny.

In the case of the female parent this hypothesis does not satisfy the positive relationships between mean length in the upper mode and both maternal smolt age and sea age at first maturity. One might conclude that the size of the female parent at spawning is relatively more important than age at smolt or sea age at first maturity. In rainbow trout, *Salmo gairdneri*, nongenetic, maternal effects make a large contribution to the variation in early growth (Bailey 1977). Gall (1972) found that rainbow trout from large eggs were larger at 25 d after hatch than those from small eggs and that the size differential increased until the conclusion of the study at 75 d after hatch. Since the energy content per unit weight of Atlantic salmon eggs is constant (Glebe et al. 1979), larger eggs will contain more nutrient material (in absolute terms) than smaller eggs. In three of the river strains of Atlantic salmon used in this study there was a direct correlation between female length and egg size (Glebe et al. 1979). The positive partial correlation between mean length in the upper mode and maternal sea age at first maturity may be an expression of maternal effects caused by variation in the quantity of nutrient material transmitted with the egg.

Partial correlations between the proportion of parr in the upper mode and parental smolt age and sea age at first maturity were only moderate for each year-class. Yet, when both year-classes were combined the relative importance of the maternal influence was increased and clearly dominant. This alludes to the presence of important maternal and environmental influences in addition to the genetic effects reported by Thorpe and Morgan (1978).

The proportion of parr in the upper mode was directly related to mean smolt age and inversely associated with mean sea age at first maturity of both parents. This means that parents that spend relatively longer periods in freshwater and shorter intervals at sea will tend to produce greater proportions of parr in the upper mode. Since the parr in the upper mode smolt earlier and spend a shorter period of time in freshwater than those in the lower mode, this result appears to be inconsistent with the concept of a genetic influence on age at smolting and Ritter's (1975) observations concerning the inverse relationship between smolt age and sea age at first maturity. With the exception of maternal smolt age, the conditions that favor relatively greater proportions of parr in the upper mode conflict with those which tend to produce relatively larger fish in the upper mode. These observations may be related to the precocious maturation of male parr.

Precocious males were observed most frequently in the upper tail of the lower modes and, to a lesser extent, in the lower tail of the upper modes (Fig. 2). Male parr that mature precociously may initially be among the faster growing individuals in the population (Glebe et al. 1978). This suggests that a minimum size must be reached before maturation can occur. In order for mature parr to participate during spawning, maturation must be synchronous with that of the larger migrating salmon, and the "decision" to mature must be made early in the growing season. Once maturation is initiated, energy previously used for somatic growth is diverted to gonadal development. This reduces the growth rate of maturing males and allows immature individuals to become relatively larger. Because mature males were found in both size modes, the size threshold for precocious maturation must be lower than that for early smolting and the "decisions" to mature as parr and to smolt early must be made independently.

In both year-classes the proportion of mature males exerted little or no influence on the mean length of parr in either size mode. The partial correlations between the proportion of individuals in the upper mode and proportion of mature males in the population, on the other hand, were large in both years, but of opposite signs (Table 3). In the 1975 year-class relatively higher proportions of mature males tended to be found in populations with relatively lower proportions of individuals in the upper mode. The opposite relationship was observed in the 1976 year-class. Both mean length of fish in the upper modes and proportion of mature male parr in the populations were greater in the 1975 than the 1976 year-class. Since the strains and crossing procedures were identical in both years, one can assume that any genetic influences on growth and early maturation would have been similar and that these differences were largely the result of environmental factors such as variation in rearing tank densities.

In the 1975 year-class, the proportion of mature males exceeded 10% of the total in 13 of 16 strains. In the 1976 year-class only 5 of 16 strains had more than 10% mature males. Furthermore, the proportion of mature males in 5 of the 1975 year-class strains equalled or exceeded the maximum of 22% observed in the 1976 year-class and 9 of the 1976 values were lower than the minimum of 5% obtained in the 1975 year-class. Thus the minimum values of the 1975 year-class tended to overlap the maximum values of the 1976 year-class. In both years, the strains within this overlapping zone (8–15% mature males) also contained the largest proportion of individuals in the upper size mode (Fig. 5). This indicates that optimum growth, leading to the maximum production of 1-yr smolts, occurred when 8–15% of the population matured precociously.

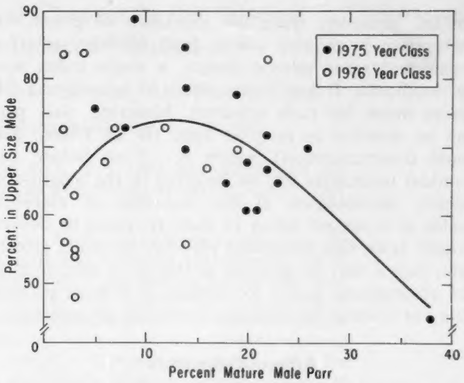


FIG. 5. Curvilinear relationship between the proportion of individuals in the upper size mode and the proportion of mature male parr in the sample of hatchery-reared Atlantic salmon.

According to the threshold size hypothesis, when very few male parr reach the size requirement for precocious maturation, a subsequent low proportion of the total population would be expected to achieve the minimum size necessary for early smolting. Conversely, if a large proportion of male parr exceed the size threshold for precocious maturation then one might also predict that a large proportion of the total population would smolt early. However, since the maturation process slows the growth rate, many of the maturing males would not reach the minimum size for early smolting. Thus, correlations between proportions of mature male parr and percentage of individuals in the upper size mode can be negative when the proportion of mature males is high, as was observed in the 1975 year-class, and positive when the incidence of mature males is low, as occurred in the 1976 year-class.

The results indicate that parental smolt age and sea age at first maturity are related to both the mean growth and smolting age of their progeny. With careful broodstock selection it should be possible to produce salmon parr that grow more rapidly and have a greater incidence of 1-yr smolts than would be expected by random mating. An additional aspect of the study suggests the possibility of indexing the incidence of precociously mature male parr with the optimum growth rate which will yield the maximum proportion of 1-yr smolts. However, since the incidence of precocious maturation is variable among both hatchery environments and some salmon strains, a single index may be insufficient. It may be necessary to determine a different index for each situation. Maturing male parr can be detected as early as June (B. D. Glebe, personal communication) which is ~2 mo before any bimodal tendencies can be detected in the length–frequency distributions. If the incidence of maturing males in a sample taken in June is found to deviate greatly from that associated with the optimum growth rate, then it may be possible, at this early date, to alter the environment either to increase growth or perhaps delay or reverse the maturation process, as necessary.

Acknowledgments

We thank Drs G. B. Ayles, R. W. Blake, B. D. Glebe, S. N. Messieh, and R. H. Peterson for their constructive reviews of earlier drafts of this manuscript.

Bailey, J. K. 1977. Intra-strain inheritance of growth and survival in rainbow trout, *Salmo gairdneri* Richardson, reared in two hatchery and two prairie winterkill lake environments. M.Sc. thesis, Univ. Manitoba, Winnipeg, Man. 133 p.

Cassie, R. M. 1954. Some uses of probability paper in the analysis of size frequency distributions. Aust. J. Mar. Freshwater Res. 5: 513–522.

Elson, P. F. 1957. The importance of size in the change from parr to smolt in Atlantic salmon. Can. Fish-Cult. 21: 1–6.

Friars, G. W., J. K. Bailey, and R. L. Saunders. 1979. Considerations of a method of analyzing diallel crosses of Atlantic salmon. Can. J. Genet. Cytol. 21: 121–128.

Gall, G. A. E. 1972. Phenotypic and genetic components of body size and spawning performance. *In* R. W. Moore [ed.] Progress in fishery and food science. Univ. Washington Publ. in Fisheries, New Series, Vol. V, Univ. Washington, Seattle, WA.

Glebe, B. D., R. L. Saunders, and A. Sreedharan. 1978. Genetic and environmental influence in expression of precocious sexual maturity of hatchery-reared Atlantic salmon (*Salmo salar*) parr. Can. J. Genet. Cytol. 20: 444 (Abstr.).

Glebe, B. D., T. D. Appy, and R. L. Saunders. 1979. Variation in Atlantic salmon (*Salmo salar*) reproductive traits and their implications in breeding programs. ICES C.M.1979/M:23.

Ritter, J. A. 1975. Relationships of smolt size and age with age at first maturity in Atlantic salmon. Tech. Rep. Ser. No. Mar./F. 75.5, Res. Dev. Branch, Maritimes Reg., Environ. Can. 7 p.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods, 6th ed. Iowa State Univ. Press, Ames, IA.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry: the principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco, CA.

Thorpe, J. E. 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish Biol. 11: 175–184.

Thorpe, J. E., and R. I. G. Morgan. 1978. Parental influence on growth rate, smolting rate and survival in hatchery reared juvenile Atlantic salmon, *Salmo salar*. J. Fish Biol. 13: 549–556.

Sedimentological and Biological Changes in the Windsor Mudflat, an Area of Induced Siltation

TED R. TURK,[1] MICHAEL J. RISK, ROY W. M. HIRTLE,[2] AND ROSS K. YEO[3]

Department of Geology, McMaster University, Hamilton, Ont. L8S 4M1

TURK, T. R., M. J. RISK, R. W. M. HIRTLE, AND R. K. YEO. 1980. Sedimentological and biological changes in the Windsor mudflat, an area of induced siltation. Can. J. Fish. Aquat. Sci. 37: 1387–1397.

The causeway-induced mudflat at Windsor, Nova Scotia, was studied to determine the effect of rapid sedimentation on the sedimentological and biological characteristics of an intertidal mudflat. Compared to typical Minas Basin mudflats, the Windsor flat has high water content, (50% vs. 30% wet wt, approximately) small grain sizes, and elevated organic carbon content (0.82% vs. 0.24% dry wt). The two principal infaunal species of the Windsor flat, the amphipod *Corophium volutator* and the clam *Macoma balthica*, are less abundant than in nearby typical flats ($\sim$840 vs. $\sim$13 000/m^2 and $\sim$640 vs. $\sim$1500/m^2, respectively), while the polychaete *Heteromastus filiformis* is particularly abundant at Windsor. *Macoma balthica* at Windsor grow faster and have a shorter lifespan ($\sim$6 yr vs. $\sim$13 yr) than at other Minas Basin flats. Compared to other Minas Basin *Macoma*, the weight of Windsor *Macoma* increases more slowly with increasing shell size, possibly an adaptation to the more fluid Windsor sediments.

These sedimentological and biological characteristics exist at Windsor nearly 10 yr after the start of flat development and show little tendency to approach typical mudflat characteristics. Our results indicate, therefore, that secondary productivity of *Macoma* and *Corophium* could be reduced by about two-thirds for at least a decade in existing Minas Basin mudflats that are covered by Windsor-type sediments as a result of the construction of a tidal power dam.

Key words: Bay of Fundy, tidal power, sedimentation, water content, organic carbon, infauna, *Corophium volutator*, *Macoma balthica*, growth, mortality

TURK, T. R., M. J. RISK, R. W. M. HIRTLE, AND R. K. YEO. 1980. Sedimentological and biological changes in the Windsor mudflat, an area of induced siltation. Can. J. Fish. Aquat. Sci. 37: 1387–1397.

Une étude de la vasière produite par la levée de Windsor, en Nouvelle-Écosse, a été effectuée dans le but de déterminer l'effet d'une sédimentation rapide sur les caractéristiques sédimentologiques et biologiques d'une vasière intertidale. Comparée aux vasières typiques du bassin des Mines, la vasière de Windsor a une forte teneur en eau (50% vs 30% en poids humide, approximativement), des grains de petites dimensions et une haute teneur en carbone organique (0,82% vs 0,24% en poids sec). Les deux principales espèces que contient la vasière de Windsor, l'amphipode *Corophium volutator* et la petite macoma *Macoma balthica*, sont moins abondantes que dans les vasières typiques avoisinantes ($\sim$840 vs $\sim$13 000/m^2 et $\sim$640 vs $\sim$1500/m^2 respectivement), alors que le polychète *Heteromastus filiformis* est particulièrement abondant à Windsor. *Macoma balthica* croît plus rapidement et a une durée de vie plus courte ($\sim$6 ans vs $\sim$13 ans) à Windsor que dans les autres vasières du bassin des Mines. Le poids des petites macomas de Windsor augmente plus lentement avec l'accroissement de taille de la coquille que les autres *Macoma* du bassin des Mines, possiblement une adaptation aux sédiments plus fluides de Windsor.

Ces caractéristiques sédimentologiques et biologiques existent à Windsor près de 10 ans après le début de formation de la vasière, et l'on voit peu de tendance d'une évolution vers les caractéristiques des vasières typiques. Nos résultats démontrent donc que, dans les vasières du bassin des Mines recouvertes de sédiments de type Windsor à la suite de la construction d'un barrage en vue du harnachement de l'énergie marémotrice, on devrait s'attendre que la productivité secondaire de *Macoma* et de *Corophium* soit réduite des deux tiers environ pendant au moins une décennie.

Received March 12, 1980 — Reçu le 12 mars 1980
Accepted May 28, 1980 — Accepté le 28 mai 1980

---

[1] Present address (T.R.T.): Tetra Tech. Inc., 630 N. Rosemead Blvd., Pasadena, CA 91107, USA.
[2] Present address (R.W.M.H.): Department of Biology, Dalhousie University, Halifax, N.S.
[3] Present address (R.K.Y.): Department of Geology, Rice University, Houston, TX.

Printed in Canada (J6003)
Imprimé au Canada (J6003)

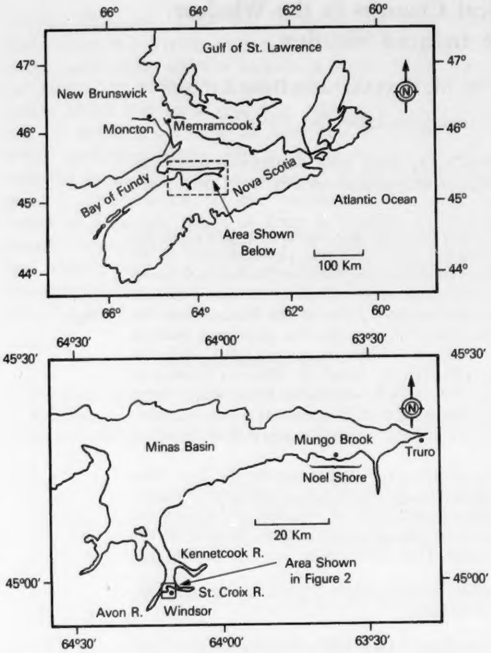


FIG. 1. Map of study areas. *Top*: The Maritime Provinces and the Bay of Fundy, showing Moncton and Memramcook, New Brunswick. *Bottom*: The Minas Basin, showing Windsor, the Noel Shore, and Mungo Brook.

THE upper reaches of the Bay of Fundy experience some of the largest tidal ranges in the world. In the Minas Basin (Fig. 1), the average difference between low and high tide levels is about 12 m. This results in extensive intertidal regions which are 2 km wide in places and have a total area of ~400 km^2 (Thomas 1977). These intertidal areas exhibit a variety of substrates: rock outcrops, and bars and flats of sand and mud.

The intertidal mudflats in the Minas Basin support a biological community dominated by deposit feeders and resembling in many respects Petersen's *Macoma balthica* community (Petersen 1924). Species diversity is among the lowest reported for marine benthic areas (Yeo and Risk 1979). In addition, population densities are very high. For example, the two most abundant species, the amphipod *Corophium volutator* and the small bivalve *M. balthica*, have mean densities of about 10 000/m^2 and 1200/m^2, respectively, and reach maxima of about 60 000/m^2 and 4000/m^2. Secondary productivity is very high as well (Yeo 1978).

Discussion of the development of tidal power in the upper reaches of the Bay of Fundy has stimulated interest in potential environmental effects (Daborn 1977). One of the major expected effects of the construction of a tidal power dam is deposition of suspended sediment, due to reduced tidal energy, in both subtidal and intertidal areas of the upper reaches (Amos 1977; Greenberg 1979).

The purpose of this study was to determine the effect of rapid sedimentation on Fundy intertidal mudflat communities. We hoped to do this by studying mudflats that accreted rapidly near causeways that were built across three rivers flowing into the head of the Bay: (1) Petitcodiac River at Moncton, N.B. (causeway built 1969); (2) Memramcook River at Memramcook, N.B. (1973); and (3) Avon River at Windsor, N.S. (1970) (Fig. 1). Since these causeways and the mudflats they produced were of varying age, we intended to estimate the rate at which communities in such induced flats approach a typical mudflat community. This approach had to be abandoned when sampling in the Moncton and Memramcook flats in the summer of 1979 revealed no benthic macrofauna (Turk 1979).

We were able to achieve our purpose, however, by studying the infaunal community of the Windsor mudflat (Fig. 2) from 1976 to 1979. Mud began accumulating seaward of the Windsor causeway soon after construction, and by 1976 the flat measured ~650 m × 700 m and contained about two million cubic feet (60 000 m^3) of sediment (Amos 1977). The flat has accreted 5 to 14 cm/mo to a height of 4 m (Amos 1977). This flat provided an opportunity to study not only the effect of sedimentation on infaunal organisms, but also sedimentological changes in an induced flat.

Areas of induced sedimentation have been studied by others, especially in the Netherlands (Terwindt 1975; Wolff 1967, 1968, 1973), but no one has investigated in any detail the benthic ecology of a mudflat with the sediment characteristics of the Windsor mudflat.

We discuss here the grain size, water content, and organic carbon content of the sediments of the Windsor flat, in addition to the abundance of the principal infaunal species, the bivalve *M. balthica*, the amphipod *C. volutator*, and the polychaete *Heteromastus filiformis*.

Methods and Materials

The Windsor mudflat was sampled during the summer of 1976, 1977, 1978, and 1979 (Table 1). For comparison, data were used from our larger study of more typical Minas Basin intertidal mudflats along the Noel Shore, N.S., particularly the flat near the mouth of Mungo Brook (Fig. 1). The Mungo Brook site was chosen because it was representative of Minas Basin flats and because data were available from the site from 1976 to 1979. Noel Shore sampling and treatment of samples were the same as Windsor low tide sampling.

Windsor sampling stations were located at regular intervals in the flat, away from drainage channels and flat margins. The difficulty of moving about the flat made repeated sampling at accurately located stations infeasible. Sedi-

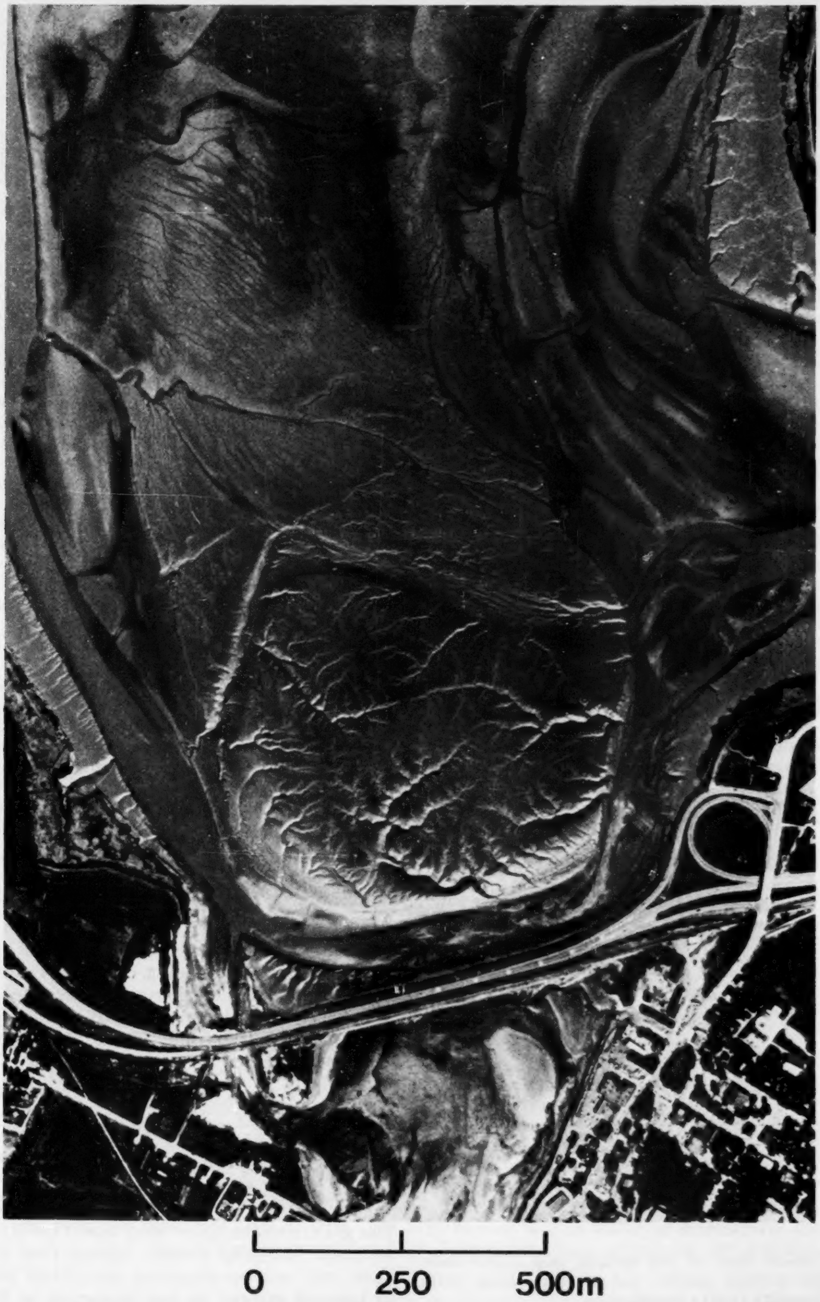


FIG. 2. Aerial photo of the Windsor Causeway and mudflat. North is towards the top. Summer 1976.

TABLE 1. Dates and types of sampling in the Windsor mudflat.

Date		Number of stations	Grain size	Water content	Carbon content	Fauna samples
Early August, 1976	Low tide, sediment cores	10	1 at each station	2 at each station	—	2 at each station
July 19–Aug. 14, 1977	Low tide, sediment cores	30	"	"	—	3 at each station
Early May, 1978	High tide, Eckman grab	16	"	"	1 at each station	—
May 24, 1978	High tide, Eckman grab	9	"	"	"	—
July 5, 1978	High tide, Eckman grab	15	"	"	"	—
July 17, 1979	Low tide, sediment cores	8	"	"	—	5 at each station

ments usually were sampled at low tide with 5-cm diameter cores. Some high tide sediment sampling was done with a small Eckman grab. Analysis for grain size, water content, and organic carbon was done on the top 2 cm of sediment from each station. Grain size analysis used sieving for material coarser than 62 μm (4.0 ϕ) and standard pipette analysis for finer material, as outlined in Folk (1968) and Carver (1971). Prior to size analysis, samples were treated with H_2O_2 to remove organics. Calgon was used as the dispersant in pipette analysis. Water content of sediment was calculated as a percentage of the total wet weight of the samples. Samples were dried to a constant weight at 110°C. Organic carbon content was determined using a LECO WR-12 carbon determinator, after removing carbonates with HCl.

We sampled animals at low tide by pushing a 16-cm diameter core into the sediment to a depth of 15 cm. (Species of interest rarely occur below this depth.) The core samples were strained through 1.0-mm mesh to separate macrofauna from sediment, and animals were counted by species. All *M. balthica* were measured from umbo to shell margin (here called shell width) to the nearest millimetre. At Mungo Brook, *Macoma* were aged by counting annual growth lines. Field observations show that the distinctly heavy lines on Minas Basin *Macoma* are in fact annual (Yeo 1978). In addition, counting rings on the shell surface has been shown to agree well with counting lines in thin shell sections (Yeo unpublished data). At Windsor, *Macoma* were aged according to their location in a detailed size–frequency distribution, where age-class peaks were discernible. This method was not possible at Mungo Brook where slower growth made age-class peaks indistinguishable.

Means in this paper were compared by *t*-tests. Tests for homogeneity of slope and elevation among regression lines were made by analysis of covariance methods. If nonhomogeneity was indicated, multiple comparisons were made with the Newman–Keuls multiple range test. Percentages in the ranges 0–30% and 70–100% were transformed to arcsine before any parametric analysis.

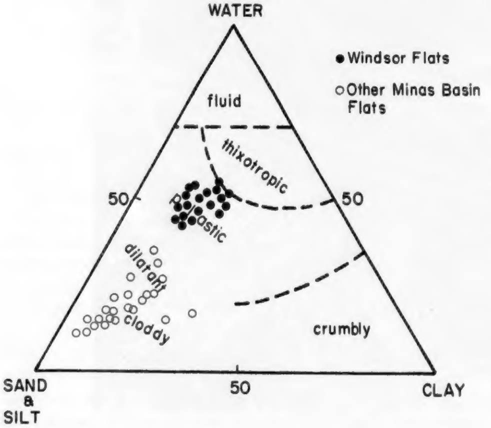


FIG. 3. Physical state of the surface (top 2 cm) sediments of the Windsor mudflat and of other Minas Basin mudflats, Boswell's (1961) classification.

Results and Discussion

SEDIMENT PROPERTIES

By comparison to typical Minas Basin mudflats, the Windsor flat has smaller grain sizes and greatly elevated water content (Fig. 3). As a result, Windsor shear strengths are significantly lower (Yeo 1978).

Overall water content of the Windsor surface sediments changed little from 1976 to 1979, but grain size tended to decrease:

	Mean water content, %	Mean grain size, ϕ
1976	47.5	5.72
1977	47.9	7.01
1978	44.3	6.37
1979	48.6	8.69

The grain size change is what would be expected in a vertically accreting mudflat approaching mean high water level and accumulating the coarser sediment on its seaward margin, as was happening at Windsor in

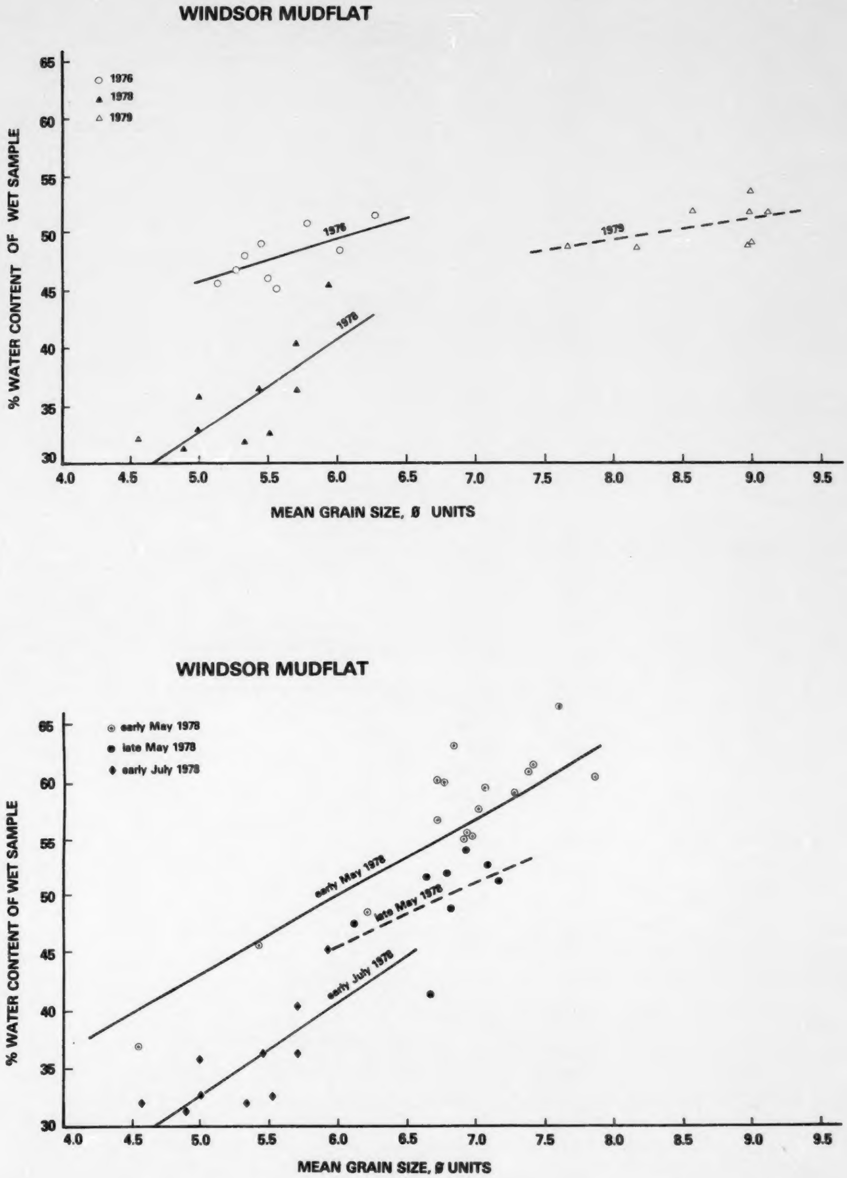


FIG. 4. Water content vs. mean grain size for the surface sediments of the Windsor mudflat. *Top*: Midsummer samples from 1976, 1978, and 1979. Text table data for 1978 differ because they are means of samples taken throughout the summer. *Bottom*: At three times during the summer of 1978. Grain size decreases toward the right. Individual data were not available for 1977.

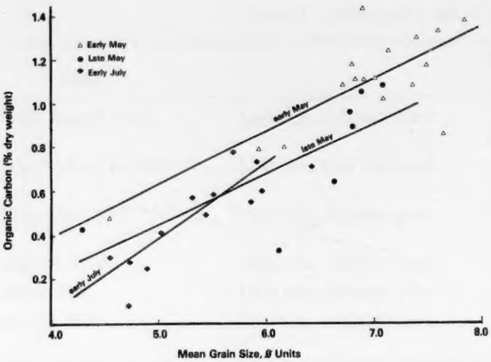


FIG. 5. Organic carbon content vs. mean grain size for the Windsor surface sediments at three times during summer 1978.

1979 (C. L. Amos, personal communication; Hirtle unpublished data).

Since the water content of sediment is influenced by grain size, plotting the former vs. the latter gives a clearer picture of water content dynamics. Midsummer water content was significantly related to grain size in 1976 and 1978 (Fig. 4, top). These two lines differ significantly ($P < 0.01$) in elevation (level) and do not differ in slope. This indicates that significant dewatering took place over a 2-yr period, amounting to a decrease in water content of about 10% in silt-sized sediments. It is not clear whether this trend continued from 1978 to 1979: percentage of water content increased, and this was accompanied by, and may be a result of, a decrease in grain size. In 1979, water content and grain size were not related.

Loss of water from the surface sediments appears to occur during summer, as shown for 1978 in Fig. 4, bottom. The regression is significant ($P < 0.01$) for early May and early July. These two lines differ in elevation ($P < 0.01$) but not in slope, indicating water loss. Elsewhere, this has also been found to occur subtidally (Yingst and Rhoads 1978).

ORGANIC CARBON

The organic carbon content of the Windsor surface sediments was determined for early May, late May, and early July, 1978. On each date, organic carbon content and mean grain size were significantly related ($P < 0.05$) (Fig. 5). This relationship has been frequently observed and several explanations have been proposed (Newell 1979), including that fine sediment provides more area per volume than coarse sediment on which microorganisms can grow. However, we cannot be sure that is the case here. From May to July, 1978, organic carbon content of the surface sediments decreased, and mean grain size increased (Fig. 5). The lines do not differ in slope or elevation, indicating no change in organic carbon content that cannot be accounted for by change in grain size. The summer organic carbon pattern at Windsor is thus different from that in typical Minas Basin mudflats, where organic carbon increases steadily during the summer, peaking in August, with grain size essentially constant (Yeo 1978). In these typical flats, much of the organic carbon in the sediments is believed to be produced in situ by benthic microflora (Yeo and Risk 1979).

TABLE 2. Densities of principal infaunal species at the Windsor mudflat and in a typical mudflat at Mungo Brook, from 1976 to 1979. Standard deviations and sample sizes are shown.

	Windsor mudflat, no./m²		
	Aug. 1976	July 1977	July 1979
Macoma balthica	1054±129 $n=20$	687±255 $n=33$	172±55 $n=40$
Corophium volutator	1631±665 $n=20$	385±615 $n=33$	501±345 $n=40$
Heteromastus filiformis	459±117 $n=20$	368±482 $n=33$	124±88 $n=40$
	Mungo Brook, no./m²		
	Aug. 1976	Aug. 1978	Aug. 1979
Macoma balthica	1537±146 $n=45$	1622±278 $n=60$	1477±3778 $n=60$
Corophium volutator	9485±4242 $n=45$	—	16691±5978 $n=60$
Heteromastus filiformis	Very low	0	0

Organic carbon values for the Windsor sediments are significantly ($P < 0.05$) higher than those from more typical Minas Basin mudflats; mean carbon content at Windsor is 0.82% dry wt (SD = 0.363%, $n =$ 37), while sediments of equivalent grain size from East Noel have a mean carbon content of 0.24% (SD = 0.150%, $n = 44$).

ORGANISM DENSITY

Macoma balthica was clearly less abundant at Windsor than at Mungo Brook (Table 2). The overall Windsor *Macoma* density was conservatively about half ($P < 0.01$) the Mungo Brook density. The difference in *Macoma* biomass between the two sites was even greater, since Windsor *Macoma* were smaller (Fig. 6). For *Corophium volutator*, the difference between the two sites is larger, with the Windsor density about an order of magnitude lower than that of Mungo Brook. *Heteromastus filiformis*, on the other hand, was more abundant at Windsor.

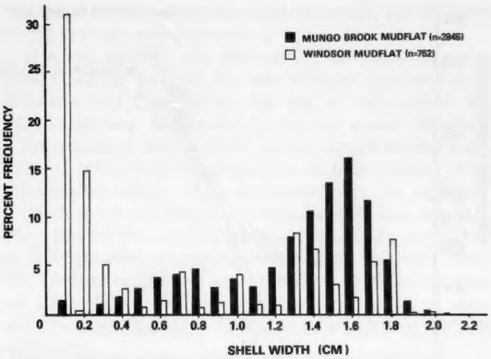


FIG. 6. Size–frequency distributions for *Macoma balthica* from Mungo Brook and the Windsor flat. 1976–79.

Macoma SURVIVORSHIP AND GROWTH

The most obvious difference between the Windsor and Mungo Brook *Macoma* size–frequency distributions (Fig. 6) is the larger proportion of very small (1–2 mm) clams at Windsor. Since these samples were taken in late summer, these small *Macoma* represented individuals that had settled as spat earlier in the season, here called the 0 age-class. Their higher abundance at Windsor indicated either greater spat settlement or better survival of spat of nearly metamorphosed clams, or both. Greater settlement may be due to the flat's location at the end of a long, linear estuary making it a site of particle (including *Macoma* spat) deposition. Good survival of young clams could be due to low competition with, or predation by, the impoverished deposit-feeding community.

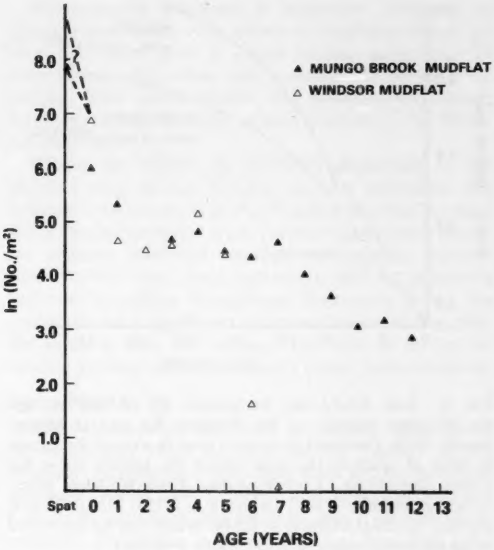


FIG. 7. Survivorship of *Macoma balthica* at Mungo Brook and at Windsor, as ln(no./m²) vs. age. The lower broken line would be spat survivorship rate if recruitment at Windsor were the same as at Mungo Brook. The upper line indicates Windsor recruitment if spat survivorship at Windsor were the same as at Mungo Brook. Data from 1976.

The survival of *Macoma* at Windsor and Mungo Brook can best be compared by plotting log of density versus age for the two sites (Fig. 7). Figure 8 shows that there is good correspondence between our identification of the age-class peaks in the detailed Windsor *Macoma* size distribution, which yielded the Windsor survivorship data, and estimated size at age from independent growth data (Fig. 9). Although age-class 0 *Macoma* were more abundant at Windsor, severe mortality in the next year there reduced the abundance of age-class 1 to well below that for Mungo Brook (Fig. 7). *Macoma* survival was high for the next few years at both sites. *Macoma* survival continued high until about the 7th yr, but few individuals older than 5 yr were found at Windsor. This seems to indicate shorter lifespan for *Macoma* at Windsor. The Fig. 8 data are from 1976, when the flat itself was only about 6 yr old. Thus one might not expect to find *Macoma* more than 5 or 6 yr old, regardless of mortality rate. However, a 1979 sample of 88 (unfortunately unaged) *Macoma* included no clams over 2.0 cm in width (length 2.4 cm), indicating either slowed growth, or more likely, a real maximum lifespan of about 6 yr at Windsor. At Mungo Brook, *Macoma* mortality rate appears to increase at about 7 yr, and maximum lifespan is 12 or 13 yr.

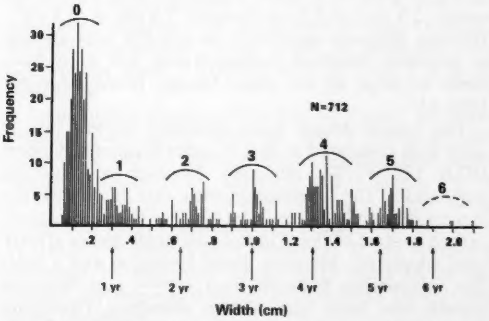


FIG. 8. Detailed size–frequency distribution of *Macoma balthica* from the Windsor flat, 1976. Age-class peaks are shown with brackets. Mean size at various ages, predicted from growth data (Fig. 9), are shown with arrows on horizontal axis.

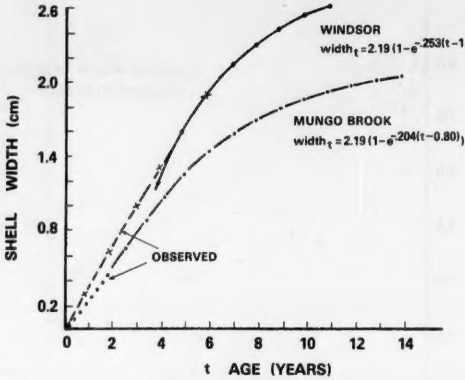


FIG. 9. Best Brody–van Bertalanffy fits of size vs. age for *Macoma balthica* at the Windsor flat and at Mungo Brook, 1976. Observed data were used to extend the curves to time of spatfall, the area where the growth curve becomes inaccurate. Corresponding Ford–Walford plots: Windsor, $\text{width}_{t+1} = 0.81\,(\text{width}_t) + 0.556$; Mungo Brook, $\text{width}_{t+1} = 0.84(\text{width}_t) + 0.344$. Width can be converted to length using length $= 1.193$ width $+ 0.0343$.

The life span at Windsor and Mungo Brook bracket most of those reported for *Macoma* (Vogel 1959; Lavoie et al. 1968; Gilbert 1973; Green 1973), although a few populations are longer (Segerstrale 1960) or shorter (Burke and Mann 1974) lived.

Shell growth rates were determined for Windsor and Mungo Brook *Macoma* by measuring distances between successive growth lines. Figure 9 is the resulting Brody–von Bertalanffy fits of shell size versus age. Windsor *Macoma* grew faster than Mungo Brook *Macoma* and showed less attenuation of growth with increasing age and size. Windsor *Macoma* are thus predicted to grow to a larger maximum shell size by the model, 2.9 cm vs. 2.2 cm (length: 3.5 cm vs. 2.7 cm). Windsor *Macoma* apparently do not live long enough to approach predicted maximum size, but many have shells as large as the older Mungo Brook *Macoma* (Fig. 6).

The typical Minas Basin *Macoma* growth rate is quite high compared to that of other locations (Gilbert 1973; Yeo 1978), especially for such a long-lived population. The Windsor growth rate is among the highest reported. This may be because the high organic carbon content of the Windsor sediments means greater food supply for *Macoma*. Food limitation and a positive relationship between food supply and *Macoma* growth rate have been shown elsewhere (Beukema et al. 1977), and there is some evidence for this in the Minas Basin as well (Tunnicliffe and Risk 1977).

In solid objects of the same shape, weight increases as the cube of linear size. A reduced rate of weight increase with increasing size has been proposed by Thayer (1975) as an adaptation to reduce sinking in bivalves living in soft substrates. Figure 10 is a log–log plot of mean total animal wet weight vs. mean shell width for *Macoma* samples from Windsor and from typical Minas Basin mudflats along the Noel Shore, including Mungo Brook. At both sites, the relationship is significant ($P < 0.01$). Weight increases less rapidly ($P < 0.01$) at Windsor (slope 1.23), where sediments are more fluid, than at Noel Shore (slope 2.24). A slope of 3.0 means weight increases as the cube of size. Our slopes are lower than those reported for bivalves by Thayer (1975).

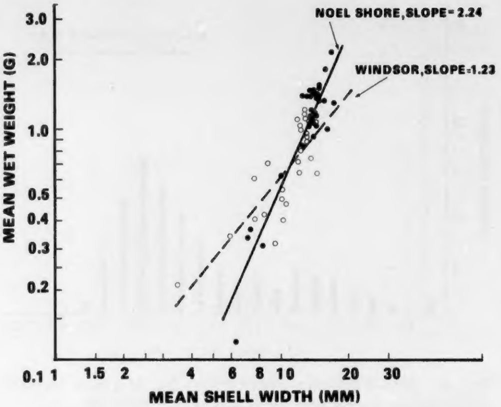


FIG. 10. Mean total animal wet weight vs. mean shell width for samples of *Macoma balthica* from the Windsor flat and from typical mudflats along Noel Shore, 1978.

General Discussion

Since Windsor and the rest of the Minas Basin have essentially the same sediment source, sediment type, and species pool, one might expect the flat to attain eventually the characteristics of a typical Minas Basin mudflat. We have no evidence to show that is occurring. The possibility that the Windsor flat is stabilizing at unique sedimentological and biological characteristics determined by local hydrographic conditions can best be judged in a few more years.

Our data generally indicate that the water content of the Windsor surface sediments is changing little, if at all. Although the flat is nearly 10 yr old, these sediments are still much more fluid than those of typical Minas Basin flats. Changes in the difficulty of walking on (or in) this flat suggest that it has compacted and dewatered at depth. In 1975, sinking to waist level was common, and the going was very difficult. By 1979, movement about the flat was much easier, with the human penetrometer usually sinking to knee or mid-thigh level. Sampling at depth, then, may be required to get a complete picture of sediment changes in this flat. However, the sediment properties of the upper few centimetres, where the infauna occur, are of overriding ecological importance.

It seems unlikely that difficulty of colonization could have much to do with the low Windsor abundance of *Macoma* and *Corophium*; the flat is well within the tidal range and dominated by marine water. Mudflats a few hundred metres away at the mouth of the Kennetcook River have high densities of both species (Yeo unpublished data). High mortality rates are probably a much more significant problem, and in fact the data show this for *Macoma*. Predation is probably not higher at Windsor than on typical Minas Basin mudflats. Flatfish feeding traces are less numerous at Windsor than on other flats, and shorebirds are less common, possibly because they have difficulty in walking in the soft sediments.

The rapid accretion of the sediments is the most likely cause of high mortality rates. Nichols et al. (1978) and Kranz (1974) have shown catastrophic sedimentation to cause mortality of infauna, apparently due to smothering. Sedimentation at Windsor is more chronic than catastrophic, and for infauna the problem of maintaining position near the surface in the resulting fluid sediments is probably more serious than smothering. Unusually soft sediments elsewhere in the Minas Basin have low numbers of *Macoma* (Risk and Moffatt 1977). For *Corophium*, burrow maintenance is likely to be an additional problem.

That support in the Windsor sediments is a problem for *Macoma* is supported by the pattern of slow weight increase of Windsor *Macoma* already discussed. This difference in growth could be due to one or a combination of factors, the most likely of which relate to the softness of the Windsor sediments: (1) variation in the size–weight relationship in *Macoma* and consequent differential mortality, with "heavy" clams tending to sink and die, leaving "light" ones for us to sample; (2) a "light" race of *Macoma* in the Bay of Fundy, adapted to soft mud; (3) direct physiological response to Windsor conditions. de Wilde (1975) found that temperatures above 15°C put *Macoma* into metabolic imbalance, due to increased oxygen consumption and reduced feeding. This was especially true for large *Macoma*, and resulted in tissue resorption and weight loss. Since water and air temperatures above 15°C are the rule at Windsor in the summer, metabolic imbalance, exaggerated by increased activity from maintaining position near the oxygenated sediment surface, could be occurring in large Windsor *Macoma*, giving them a low weight/size ratio.

The idea of metabolic imbalance conflicts with our earlier use of high food supply as an explanation for rapid shell growth in Windsor *Macoma*. However, rapid shell growth could still be an effective response to soft mud under marginal metabolic conditions, if the weight/size ratio were decreased significantly as a result. A better evaluation of these possibilities will require more work on shell and soft part growth of *Macoma* and their relation to Windsor conditions.

Heteromastus filiformis is unusually abundant at Windsor and seems well suited to conditions there. Its long, thin shape gives it a high surface/mass ratio. In conjunction with setae, this reduces the problem of sinking. This would enable this upside-down deposit feeder to exploit the high organic content of the Windsor sediments.

Winter ice effects are probably important to the physical state of the Windsor surface sediments. Ice buildup is more severe on the Windsor flat than on most Minas Basin intertidal areas (Hirtle unpublished data). Ice disrupts intertidal mudflats by gouging furrows which collect fine, fluid sediments, and by removing sediment by rafting (Knight and Dalrymple 1976). Ice could also be a significant additional cause of mortality for infauna near the surface. The role of ice in the benthic ecology of Bay of Fundy needs more attention.

TIDAL POWER

The relevance of our study to possible tidal power environmental effect depends on the validity of the Windsor mudflat as a post-tidal dam sedimentological analogue. Amos (1977, 1979) and Risk (1979) have tentatively proposed this analogy, but it is difficult to evaluate, partly due to uncertainties of final dam location and design. The best model to date on the sedimentological effects of the construction of a dam in the Minas Basin, however, predicts accelerated sedimentation in the part of the Minas Basin intertidal where mudflats and salt marshes now exist (Greenberg 1979). Our results show that this could have a major effect on the productivity of these areas. In typical Minas Basin mudflats, *Macoma* and *Corophium* account for almost all of the secondary productivity, with a combined productivity of the two species of about 21 g dry wt$\cdot$m$^{-2}\cdot$yr^{-1} (Yeo 1978). Should barrage construction produce the predicted intertidal siltation resulting in development of Windsor-type conditions over a preexisting flat, our data show that 10 yr later the drastic decrease in *Macoma* and *Corophium* population densities (~1500 to 640/m^2 and 13 000 to 840/m^2, respectively) could result in secondary productivity of about 7 g dry wt$\cdot$m$^{-2}\cdot$yr^{-1}, or a drop of two-thirds.

In the upper reaches of the Bay of Fundy, intertidal infauna, particularly *Macoma* and *Corophium*, are major food sources for large migratory shorebird populations (Hicklin 1977; Morrison 1977; Boates 1979) and for many species of fish (Risk and Craig 1976; Flemming 1979; Gilmurray 1979; Salinas 1979), some of which are economically important. If our estimated decreases in secondary productivity were to be extensive, the consequences for bird and fish populations could be serious.

Acknowledgments

This work was supported by Research Subventions from the Department of Energy, Mines & Resources, an NSERC Operating Grant, and an NSERC Strategic Grant. We are grateful to Dr Carl Amos for helpful discussions of the sedimentology of Windsor mudflat and for logistical help there. For assistance in the field and lab we thank Paulette Burns, Nan Ferguson, Martin Hewitt, Larry Hildebrand, Deborah Hunter, Katie Isbester, Ann-Kristin Lundblad, Garth Prime, Jim Smith, Verena Tunnicliffe, and Eric Zaunscherb. Michael Dickman, Peter Martini, David Scarratt, and two anonymous reviewers read an earlier version of the manuscript and made useful suggestions. We thank Lorie Cooper and Jack Whorwood for help with the illustrations; Joyce Allen, Angela Antanavicius, and Donna Block for typing; and the Nova Scotia Department of Highways for the mudflat.

Amos, C. L. 1977. The effects of tidal power structures on sediment transport and loading in the Bay of Fundy–Gulf of Maine system, p. 233–253. *In* G. Daborn [ed.] Fundy tidal power and the environment. Acadia University Press, Wolfville, N.S.

1979. Sedimentation resulting from Fundy tidal power, p. 173–179. Proc. of the Colston Symposium 30, Bristol, U.K. April 1978.

Beukema, J. J., G. C. Cadee, and J. J. M. Jansen. 1977. Variability of growth rate of *Macoma balthica* in the Wadden Sea in relation to availability of food, p. 69–77. *In* B. F. Keegan, P. O. Ceidigh, and P. J. S. Boaden [ed.] Biology of benthic organisms. Pergamon Press, Oxford.

Boates, J. S. 1979. Predator–prey interactions between migrated semipalmated sandpipers and *Corophium volutator* in the Minas Basin, Nova Scotia. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick, November 1979 (Abstr.)

Boswell, P. G. H. 1961. Muddy sediments. W. Heffer and Sons, Limited, Cambridge. 140 p.

Burke, M. V., and K. H. Mann. 1974. Productivity and production: biomass ratios of bivalve and gastropod populations in an eastern Canadian estuary. J. Fish. Res. Board Can. 31: 167–177.

Carver, R. E. [ed.] 1971. Procedures in sedimentary petrology. Wiley Interscience, New York. 653 p.

Daborn, G. [ed.] 1977. Fundy tidal power and the environment. Acadia University Press, Wolfville, Nova Scotia. 304 p.

de Wilde, P. A. W. J. 1975. Influence of temperature on the behavior, energy metabolism, and growth of *Macoma balthica*, p. 239–256. *In* H. Barnes [ed.] Proc. of the Ninth European Symposium on Marine Biology. Aberdeen Press, Aberdeen.

Flemming, J. 1979. Population estimates of *Microgadus tomcod* and *Paralichthys dentatus*, and their impact on the ecosystem. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick. November 1979 (Abstr.).

Folk, R. L. 1968. Petrology of sedimentary rocks. Hemphill's Book Store, Austin, TX. 170 p.

Gilbert, M. A. 1973. Growth rate, longevity, and maximum size of *Macoma balthica*. Biol. Bull. 145: 119–126.

Gilmurray, C. 1979. Feeding relations of some juvenile fish in Minas Basin, Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick. November 1979 (Abstr.).

Green, R. H. 1973. Growth and maturity in an Arctic intertidal population of *Macoma balthica* (Pelecypoda). J. Fish. Res. Board Can. 30: 1345–1348.

Greenberg, D. A. 1979. The effects of a tidal barrage on the deposition of suspended particulate matter in the Bay of Fundy. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick. November 1979 (Abstr.).

Hicklin, P. W. 1977. The utilization of substrates and their respective invertebrate fauna by shorebirds in the Minas Basin. Contract Rep., Can. Wildl. Ser. KL-210-6-5665: 37 p.

Knight, R. J., and R. W. Dalrymple. 1976. Winter conditions in a macrotidal environment, Cobequid Bay, Nova Scotia. Rev. Geogr. Montreal 30: 65–85.

Kranz, P. M. 1974. The anastrophic burial of bivalves and its paleoecological significance. J. Geol. 82: 237–265.

Lavoie, R., J. L. Tremblay, and G. Filtreau. 1968. Age et croissance de *Macoma balthica* a Cacouna-est dans l'estuaire de St. Laurent. Naturaliste Canadien 95: 887–895.

Morrison, R. I. G. 1977. Use of the Bay of Fundy by shorebirds, p. 187–199. *In* G. Daborn [ed.] Fundy tidal power and the environment. Acadia University Press, Wolfville, N.S.

Newell, R. C. 1979. Biology of intertidal animals, 3rd ed. Marine Ecology Surveys Limited, Faversham, Kent, United Kingdom. 781 p.

Nichols, J., G. T. Rowe, C. H. Clifford, and R. A. Young. 1978. *In situ* experiments on the burial of marine invertebrates. J. Sediment. Petrol. 48: 419–426.

Petersen, C. G. J. 1924. A brief survey of the animal communities in Danish waters. Am. J. Sci. 4(5): 343–354.

Risk, M. J. 1979. Recent changes in the benthic ecology of the Windsor flat. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick, November 1979 (Abstr.).

Risk, M. J., and H. D. Craig. 1976. Flatfish feeding traces in the Minas Basin. J. Sediment. Petrol. 46(2): 411–413.

Risk, M. J., and J. S. Moffatt. 1977. Sedimentological significance of fecal pellets of *Macoma balthica* in the Minas Basin, Bay of Fundy. J. Sediment. Petrol. 47(4): 1425–1436.

Salinas, I. 1979. Pecks Cove mudflat ecosystem study IV, feeding of intertidal fish. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick. November 1979 (Abstr.)

Segerstrale, S. G. 1960. Investigations on Baltic populations of the bivalve *Macoma balthica*. Soc. Sci. Fenn. Commentat. Biol. 23: 1–72.

Terwindt, J. H. J. 1975. Milieu-randvoorwaarden voor het gedempte getijgebied in de Oosterschelde, Rijkswaterstaat-Deltadienst, nota nr. 75–72: 30 p.

Thayer, C. W. 1975. Morphologic adaptations of benthic invertebrates to soft substrata. J. Mar. Res. 33: 117–189.

Thomas, M. L. H. 1977. Intertidal resources of the Bay of Fundy, p. 148–159. *In* G. Daborn [ed.] Fundy tidal power and the environment. Acadia University Press, Wolfville, N.S.

Tunnicliffe, V., and M. J. Risk. 1977. Relationships between the bivalve *Macoma balthica* and bacteria in intertidal sediments: Minas Basin, Bay of Fundy. J. Mar. Res. 35(3): 499–507.

Turk, T. R. 1979. Siltation from man-made structures. Fundy Environmental Studies Committee Annual Workshop, Biological Station, St. Andrews, New Brunswick. November 1979 (Abstr.).

Vogel K. 1959. Wachstumsunterbreachungen bei Lamellibranchiaten und Brachiopoden. Neues Jahrb. Geol. Palaeontol. Abh. 109: 109–129.

Wolff, W. J. 1967. Watervogeltelligen in het gehele Nederlandse Deltagebied. Limosa 40: 416–425.

1968. Een sammenvatting van de gegevens over de Kluten van het Deltagebied in de loop van het jaar. Levende Nat. 71: 35–42.

1973. Resultaat van vijf jaar steltlopertellingen op Schouwen. Limosa 46: 21–41.

YEO, R. K. 1978. Animal–sediment relationships and the ecology of the intertidal mudflat environment, Minas Basin, Bay of Fundy. M.Sc. thesis, McMaster University, Hamilton, Ont. 396 p.

YEO, R. K., AND M. J. RISK. 1979. Fundy tidal power: Environmental sedimentology. Geosci. Can. 6: 115–121.

YINGST, J. Y., AND D. C. RHOADS. 1978. Seafloor stability in central Long Island Sound: Part II. Biological interactions and their potential importance for seafloor stability, p. 245–260. *In* M. L. Wiley [ed.] Estuarine interactions. Academic Press, N.Y.

Circulation and Energetics of a Deep, Strongly Stratified Inlet

HOWARD J. FREELAND AND DAVID M. FARMER

Institute of Ocean Sciences, Sidney, B.C. V8L 4B2

FREELAND, H. J., AND D. M. FARMER. 1980. Circulation and energetics of a deep, strongly stratified inlet. Can. J. Fish. Aquat. Sci. 37: 1398–1410.

The paper compares a theoretical model of steady flow in a strongly stratified inlet with the observed behavior of Knight Inlet, British Columbia. Some aspects of the behavior compare favorably with the theory and some aspects do not. Specifically, the model does well in the limit of small interfacial friction coefficient, which is surprising as the evidence presented by Long (1975) suggests that interfacial friction should dominate the flow. Long demonstrates that the circulation in inlets of this type should adjust to maintain the interfacial Froude number at unity near the mouth. In the paper we demonstrate that Knight Inlet is not apparently being controlled in this way.

Computations of the amount of energy required to drive the circulation, the amount that can be extracted from the wind, and the amount that is being lost by the barotropic tides indicate that tidal interactions with the sill in Knight Inlet drive the mean circulation. The wind contributes little to the overall energy budget of Knight Inlet.

Key words: Knight Inlet, circulation, dynamics, energetics, internal waves, turbulence, mixing processes, entrainment

FREELAND, H. J., AND D. M. FARMER. 1980. Circulation and energetics of a deep, strongly stratified inlet. Can. J. Fish. Aquat. Sci. 37: 1398–1410.

Le présent article compare un modèle théorique d'écoulement uniforme dans un bras de mer fortement stratifié avec le comportement observé de l'inlet Knight, en Colombie-Britannique. Sous certains rapports, le comportement de ce dernier se compare favorablement avec la théorie, alors que sous d'autres, il n'y a pas concordance. Plus précisément, le modèle donne de bons résultats dans la limite d'un faible coefficient de friction interfacial, ce qui est surprenant, car, selon les données présentées par Long (1975), la friction interfaciale devrait dominer l'écoulement. Long démontre que la circulation dans les bras de mer de ce type devrait s'ajuster de façon à maintenir l'indice interfacial de Froude égal à l'unité près de l'entrée. Nous démontrons ici que l'inlet Knight ne semble pas contrôlé de cette manière.

Les calculs de la quantité d'énergie requise pour entraîner la circulation, la quantité pouvant être retirée du vent et celle perdue par les marées barotropes indiquent que les interactions de la marée avec le seuil de l'inlet Knight entraînent la circulation moyenne. La contribution du vent au budget énergétique global de l'inlet Knight est minime.

Received February 4, 1980
Accepted May 28, 1980

Reçu le 4 février 1980
Accepté le 28 mai 1980

THIS paper will examine the circulation and associated hydrography of a deep stratified inlet, and will compare the observed behavior of that inlet with the predictions of a theory of the circulation due to Long (1975). During the comparison it will become evident that the inlet divides naturally into two regions having substantially different oceanographic properties. The reason for the existence of the different regions will be discussed and this will shed some light on the source of energy that drives the general circulation of this inlet and possibly others. Current meter observations obtained from Knight Inlet will also be discussed, and it will be shown that these observations provide some verification of the energy scheme outlined.

Knight Inlet is a fjord on the mainland of British Columbia, Canada (Fig. 1), and in its general morphology is typical of fjords of British Columbia. The inlet is generally deep, greatest depth 550 m, but has a sill 75 km from the head of the inlet where depth falls to about 60 m. The sill is in the middle of a long straight reach which is connected to the head by a sinuous reach. As will be shown later, the oceanography of the straight reach is fundamentally different from that of the sinuous reach. The sides of the inlet slope downwards very steeply, and a model describing the inlet as having a rectangular cross section is a good approximation.

The general oceanography of Knight Inlet has been described elsewhere, e.g. Pickard (1961) and Pickard and Rodgers (1959), and is briefly reviewed here. Most of the freshwater enters at the head through the Klinaklini River, a smaller, but significant amount enters a few kilometres away through the Franklin

Printed in Canada (J5955)
Imprimé au Canada (J5955)

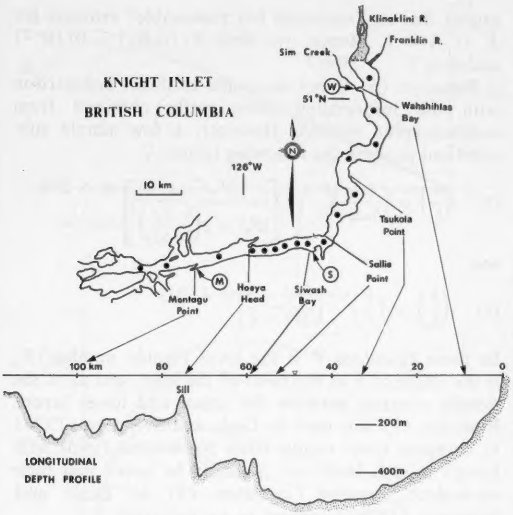


FIG. 1. Plan view and longitudinal depth profile of Knight Inlet. The locations of tide gauges are indicated by the symbols Ⓜ, Ⓢ, and Ⓦ, and of CTD stations by the symbol ●. Several locations mentioned in the text are identified on the plan and profile.

River, and very small amounts enter through creeks along the remainder of its length. The creeks do not contribute significantly to the total runoff. The mean annual river discharge is estimated to be about 400 m^3/s with a maximum in June of around 800 m^3/s. The June maximum is dominated by glacial melt water; a secondary maximum (which did not occur in 1977 because of unusual weather conditions) is related to the onset of the rainy season in November. Through the winter the runoff is small, typically 50 m^3/s.

Throughout most of the year the stratification of Knight Inlet is such that a description as a two-layer structure seems appropriate. Indeed, two theoretical analyses of the flow of two-layer estuaries have selected Knight Inlet as an archetype (Long 1975; Pearson and Winter 1978). Long described an analysis of the flow distributions permitted in a deep stratified inlet. The theory developed is applied to Oslofjord and Knight Inlet, mainly because substantial bodies of information exist for each of these cases. For the latter case, Long predicts that the depth of the upper layer close to the freshwater input should be about 21 m. This seems to be far too large; observationally, the depth appears to vary between 3 and 5 m depending on the season. The theory has been criticized by Pedersen (1978) on the grounds that interfacial shear stresses are not balanced. This is a valid criticism, but may not be important if the lower layer is deep enough that velocities remain always very small. Long does assume that the flow and horizontal pressure and density gradients in the lower layer can be neglected in the momentum equations and the solutions he obtains are dominated by friction and a steep slope of the free surface. Closure is obtained under a particular assumption regarding the entrainment process and by an outer boundary condition, chosen in this case to be Froude number = 1 at the mouth, following Stommel and Farmer (1953).

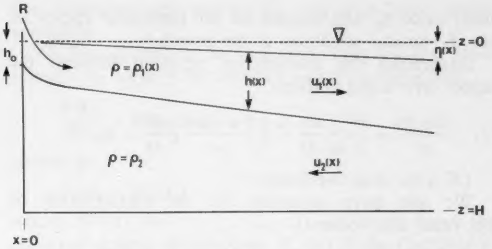


FIG. 2. Definition of symbols for the estuarine circulation model.

We will show in this paper that the hydrography of Knight Inlet is to some extent in agreement with the predictions of Long's analytical model. However, we disagree with Long's determination that the circulation is dominated by friction and a large free surface slope and we find friction to be unimportant; hence the criticism made by Pedersen (1978) is moot. The hydrography implied by Long's outer boundary condition does not occur, and the Froude number remains well below unity at the mouth.

Fjord Circulation

In this section we review certain properties of Long's model of fjord circulation and then compare them with the observed behavior of Knight Inlet.

We consider a simple model of a two-layer stratification in a flat-bottomed inlet (Fig. 2). The freshwater input, density ρ_0, is entirely at one end and has a volume flux R. The density of the upper layer is ρ_1 and variable; and the density of the lower layer is ρ_2 and fixed. As water moves seaward in the upper layer, turbulence causes water from the lower layer to mix into the upper increasing $\rho_1(x)$ and $h(x)$ (thickness of the upper layer), and work is done to increase the potential energy of water columns. Since salt is advected out in the upper layer, a deep return flow, $u_2(x)$, is required. If the depth of the fluid, H, is very large compared with $h(x)$, then $u_2(x)$ is very small and deep pressure gradients are negligible.

We will simplify Long's model somewhat by keeping the channel width, W, constant; this is a good approximation for Knight Inlet. Writing conditions at the head as:

$$\rho_1(x = 0) = \rho_0;\ h(x = 0) = h_0;\ u_1(x = 0) = u_0 = R/wh_0.$$

(Note that these are not necessarily *boundary condi-*

tions since h_0 will depend on the particular choice of the dynamical condition at the mouth.)

Integrating the momentum equation through the upper layer Long derives:

$$(1)\quad \frac{d(u_1{}^2h)}{dx} = \frac{h^2}{2}\frac{g}{\rho_0}\frac{d\rho_1}{dx} - h\frac{(\rho_2-\rho_1)}{\rho_0}g\frac{dh}{dx} - Ku_1{}^2$$

(K is the drag coefficient.)

We also have equations for the conservation of buoyancy and volume:

$$(2)\quad u_1(\rho_1-\rho_0)h + u_2(\rho_2-\rho_0)(H-h) = 0$$

$$(3)\quad u_1h + u_2(H-h) = u_0h_0 = R/W.$$

An equation for the conservation of volume in the upper layer yields a definition of the entrainment velocity u_e.

$$(4)\quad \frac{d(hu_1)}{dx} = u_e(x).$$

This set yields four equations in five dependent variables, so a further equation is needed to close the set. Long chooses to set the entrainment velocity proportional to the intensity of turbulence and inversely proportional to a bulk Richardson number, thus:

$$(5)\quad u_e(x) = \frac{K_n\sigma}{Ri} = \frac{K_n\rho_0\sigma^3}{gh(\rho_2-\rho_1)}.$$

Where σ is the root mean square turbulent velocity and K_n is a constant which Long sets equal to 0.1. Equation (5) is based on the results of numerous laboratory experiments, Kato and Phillips (1969) for example.

The proportionality of the entrainment velocity to the inverse Richardson number is open to some dispute. If we assume that u_e is proportional to $R_i{}^{-n}$, then Kantha (1975) has found, in laboratory experiments, n ranging from $\frac{1}{2}$ to 2 depending on the size of the Richardson number. However, $n = 1$ is a good choice covering a wide range of Richardson numbers.

The solution can be written in a variety of ways; Long's equation (21) can be written:

$$(6a)\quad \frac{h}{h_0} = \frac{u_1}{u_0}\left[\frac{2u_0{}^3 + \beta u_0c_0{}^2}{2u_1{}^3 + \beta u_0c_0{}^2}\right]^{(\beta+2)/3}$$

Here, β is a constant $\{\beta = s - 1$ in Long's notation$\}$ that measures the influence of friction. Specifically:

$$(6b)\quad \beta = \left(1 + \frac{K}{mK_n}\right)^{-1}$$

and where

$$m = \frac{\sigma^3\rho_0}{g(\rho_2-\rho_0)u_0h_0} = \frac{\sigma^3}{u_0c_0{}^2}$$

and where c_0 is the speed of an internal gravity wave near the head of the inlet. For flows dominated by friction, β approaches zero from above, and for frictionless flow, β is unity. K_n is believed to be around 0.1, and for reasonable estuarine type flows, m would be expected to be substantially less than unity. Long (1975) argues that an "uncertain but reasonable" estimate for K is $K = 1$. Hence, we find $K/(mK_n) = 0(10^{-2})$ and $\beta \ll 1$.

Equation (6) is not amenable to direct comparison with the information most easily obtained from oceanographic surveys. However, a few simple substitutions produce the following forms:

$$(7)\quad \left(\frac{h}{h_0}\right) = \left(\frac{h\Delta\rho}{h_0\Delta\rho_0}\right)^{\beta+1}\left[\frac{2F_0{}^2+\beta}{2F_0{}^2+\beta\left(\frac{h}{h_0}\frac{\Delta\rho}{\Delta\rho_0}\right)^3}\right]^{(\beta+2)/3}$$

and

$$(8)\quad \left(\frac{h}{h_0}\right) = \left(\frac{F}{F_0}\right)^{2/3}\left[\frac{2F_0{}^2+\beta}{2F^2+\beta}\right]^{(\beta+2)/3}$$

In these equations F is the local Froude number, F_0 is the value of F at the head of the inlet, and $\Delta\rho$ is the density contrast between the upper and lower layers. Equation (7) was used by Gade and Svendsen (1978) to compare some results from Norwegian fjords with Long's model. However, it should be noted that their equivalent equation {equation (8) in Gade and Svendsen (1978)} appears to be incorrect.

Figures 3a and 3b show how h/h_0 varies with $\Delta\rho$ and F, respectively. A specific value of F_0 has been chosen, but the behavior does not change much over the range of values F_0 is likely to take. The variation of h/h_0 is shown for a selection of values of β. For small values of F the upper layer thickness increases. For all values of β, h takes a maximum value at some value of the Froude number F_m, specifically $F_m{}^2 = \beta/(2\beta+2)$. The density of the upper layer increases steadily up to a Froude number of unity. At $F = 1$ transport in the upper layer is maximized. The Froude number cannot be forced to go from just less than unity to just greater than unity, since then some mechanism for removing salt from the upper layer and adding it to the lower layer would have to be found. Hence, Froude numbers are bounded to the region $F_0 \leq F \leq 1$. The maximum value of h/h_0 is a fairly strong function of F_0; for any value of β, the maximum value of h/h_0 increases as F_0 decreases. For the inviscid case of $\beta = 1$,

$$\max(h/h_0) = 0.42(1 + 2F_0{}^2)F_0{}^{-2/3}.$$

We will now examine some of the hydrographic data from Knight Inlet in the light of some of the properties discussed above. Between January 1977 and December 1978 hydrographic surveys of Knight Inlet were carried out at about 1-mo intervals. During summer when the runoff is extremely high, the density contrast between the upper and lower layers occurs over a very small vertical displacement and the CTD used in our surveys was unable to handle the abrupt changes in conductivity. The records initially "spiked" and then oscillated around the thermocline. We will discuss the "depth of the upper layer" of a two-layer representation of the stratification, and so to avoid ambiguity we will not consider any midsummer profiles. During the

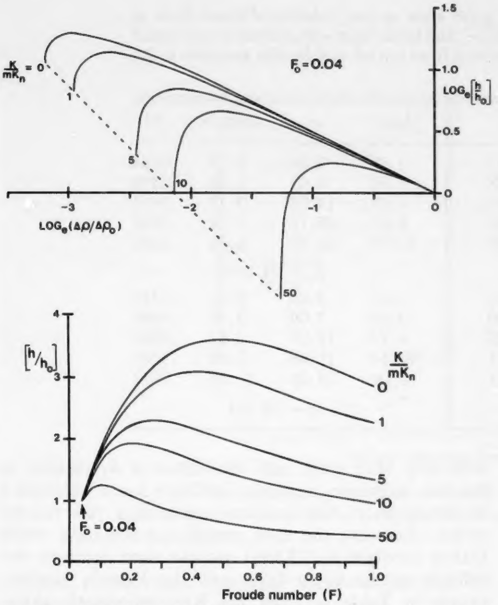


FIG. 3. Variation of (h/h_0) with density contrast $(\Delta\rho/\Delta\rho_0)$ and Froude number (F) in 3a (*upper*) and 3b (*lower*), respectively, for five different values of the parameter K/mK_n. The initial Froude number F_0 is 0.04, and within the range of values that might be encountered in Knight Inlet.

winter when the runoff is very small, the salinity of the upper layer at the head of the inlet is sufficiently high that the circulation cannot really be considered to be fjord-like in the classical sense. These two constraints restrict us to spring and fall surveys. There may be a further constraint: evidence presented in a paper by Farmer and Smith (1980) shows that the summer response to flow over the sill is in the second internal mode. Hence, two internal degrees of freedom are required to describe motions in the inlet and so a two-layer representation may be entirely inappropriate in midsummer.

As with the application of any two-layer model that seeks to represent a continuous density distribution, it is necessary to devise an objective approach that incorporates the observed density structure in the calculation of relevant parameters for the two-layer representation. There are many ways of doing this but the following procedure has the advantage of including elements of physics that appear to be relevant to the present study, namely the speed of internal waves and the available potential energy of the water column. Our approach is to compute the first mode internal wave speed c_1 and also the potential energy χ_{obs}, for the observed density distribution and equate these to the formulae appropriate to the two-layer representation.

We first determine the first mode long internal wave speed c_1 by solving the standard eigenvalue problem.

$$\frac{d^2W_n}{dz^2} + W_n(z)N^2(z)/c_n^2 = 0$$

subject to

$$W_n(0) = W_n(H) = 0,$$

where $N^2(z) = -(g/\rho_0)(d\rho/dz)$ is found from the observed density distribution, $W_n(z)$ is the distribution of the amplitude of the nth eigenfunction, and c_n is its phase speed. We retain only c_1, the speed of the first internal mode. We next compute the potential energy for the same density profile:

$$\chi_{\mathrm{obs}} = \frac{1}{H^2}\int_0^H \sigma_t(z)z\,dz,$$

where $\sigma_t = 10^3(\rho - 1)$ and H is the total fluid depth. Equating these observed values to the corresponding expressions for the two-layer representation:

$$c_1^2 = g'(\sigma_2 - \sigma_1)h(H - h)/H,$$

$$\chi_{\mathrm{obs}} = \tfrac{1}{2}\{\sigma_1 h^2 + \sigma_2(H^2 - h^2)\}/H^2$$

where $g' = g/(\rho_2 \times 1000)$. Solving for the upper layer depth h and lower layer density σ_2 yields:

$$h = (c_1^2/g')/(2\chi - \sigma_1 - c_1^2/(g'H)) \tag{9}$$

and

$$\sigma_2 = \sigma_1 + c_1^2H/\{g'h(H - h)\}. \tag{10}$$

Thus h and σ_2 can be calculated with one additional constraint. The final constraint we used was to set σ_1 to the observed value of σ_t at a depth of 2 m. As a check on the stability of the approach we calculated h and σ_2 with the observed value of σ_1 at 0, 1, 2, 3, and 4 m. The calculated values of h varied by less than 10% as long as σ_1 was not set to an observed value below the pycnocline and the calculated values of σ_2 varied very little.

For a sharp interface, such as always occurs near the head, this objective determination of upper layer thickness agrees exactly with the value that would have been picked intuitively. When a substantial amount of mixing has occurred it is no longer obvious what a subjective observer would pick, so a comparison is futile.

Later in the paper we will examine the energetics of the inlet and discuss the role of control of the circulation by the speed of long internal waves. Hence, it is important that the wave speed and potential energy be conserved in the reduction to a two-layer system.

Figure 4 shows plots of the upper layer thickness, calculated using equation (9), plotted against distance from the head of the inlet for four cruises. For reference, the sinuous reach lies between km 0 and 55, and the straight reach between km 55 and the mouth at km 101. The sill is at km 75. Clearly the data from the sinuous and straight reaches belong to different regimes.

TABLE 1. Upper layer thickness h in metres; upper layer σ_t (σ_1); velocity of upper layer u_1 in cm/s; Froude number $(Fr) = u_1/\sqrt{gh(\rho_2 - \rho_1)/\rho_1}$; and lower layer $\sigma_t(\sigma_2)$. Data are presented for the four cruises numbers 4, 9, 15, and 8, clockwise from top left and for five locations in the inlet.

$x(km)$	$h(m)$	σ_1	u_1(cm/s)	Fr	$h(m)$	σ_1	u_1(cm/s)	Fr
0	3.25	0.00	2.78	.0313	2.88	0.00	2.22	.0264
25	3.78	5.87	3.16	.0378	3.64	6.61	2.40	.0299
50	4.30	12.27	4.24	.0589	4.41	13.22	3.17	.0452
75	9.63	21.13	7.17	.1295	8.02	20.74	5.34	.0990
100	15.32	22.87	9.94	.2115	12.73	22.44	6.34	.1280
		$\sigma_2 = 24.313$				$\sigma_2 = 24.365$		
0	2.53	0.00	2.93	.0373	3.23	0.00	2.79	.0316
25	3.03	4.91	3.07	.0400	3.98	7.00	3.19	.0386
50	3.53	8.40	3.21	.0428	4.73	12.07	3.81	.0502
75	7.08	20.05	5.90	.1064	10.14	21.40	7.70	.1445
100	11.16	21.59	5.80	.1037	19.09	23.28	12.45	.2972
		$\sigma_2 = 24.393$				$\sigma_2 = 24.201$		

Two important differences are

i) The rate at which the upper layer deepens is much greater on the straight reach than on the sinuous reach.

ii) The measurement of h is much noisier in the straight reach.

The observation of a slow deepening in the sinuous reach could not have been made with a subjective method of estimating h. Both of the observations above are reproduced at other times of the year, even in February when the upper layer salinity exceeds 20‰ near the head. This behavior is also evident in a survey reported by Farmer and Smith (1978), though that survey did not extend all the way to the mouth. The bold lines in Fig. 4 are linear regressions through the points.

Before making quantitative comparisons between observations and theory, a few elementary comparisons can be made directly from Fig. 4. Firstly, Long's model predicts an upper layer thickness near the head of 21 m. Clearly we differ with this prediction. Secondly, Long's model, for any value of the friction parameter, predicts that the upper layer thickness should go through a maximum. In three of the four plots on Fig. 4 the last point observed at the mouth yields the greatest thickness measured anywhere in the inlet. The pattern of distribution of upper layer thickness expected from Long's model does not seem to be occurring. However, to know whether or not this observation is significant we need to know over what characteristic distance the maximum upper layer thickness is achieved compared with the total length of the inlet. To determine this we need to know the size of some of the uncertain parameters for Knight Inlet.

Using the regression lines sketched on Fig. 4 the interface depth was noted at five positions in the inlet, chosen arbitrarily to be at km 0, 25, 50, 75, and 100. Those values of upper layer thickness are noted in Table 1. The density of the upper layer varies smoothly with very little noise, and the values of σ_1 entered at the five reference locations in Table 1 are calculated by fitting short line segments through a few nearby points. Knowing the total runoff and the inlet width (taken constant at 2.5 km) we can then compute the velocity in the upper layer and the Froude number, shown in Table 1, from the Knudsen relationships, equations (2) and (3). Also listed in Table 1 are the values (for each cruise) of the average σ_2 measured for the lower layer. The scatter about these values among the stations in any one cruise is small, typically 0.07. Using these data we can enter points on a plot of h/h_0 versus $\Delta\rho/(\Delta\rho_0)$ as shown in Fig. 5. For reference two sets of curves have been superimposed on Fig. 5, the variation that is predicted by Long's model for each of the cruises (varying initial Froude number, F_0), and for two values of the friction parameter K/mK_n. The bold lines are the inviscid case, $K = 0$, and the broken lines are for $K/mK_n = 5$. Clearly the fit to the family of inviscid solutions is not bad, though it could be better; however, the fit to the family of viscid solutions is poor. A conservative statement might then be made that $K/mK_n < 5$. If we can estimate the denominator mK_n, then we can put an upper bound on the interfacial friction coefficient.

Using the data in Table 1 and equation (4), we can estimate the entrainment velocity over various regions of the inlet. We estimate u_e in the sinuous reach as: $u_e = \{u_1(50)h(50) - u_1(0)h(0)\}/50$ km and find $u_e = (1.5 \pm 0.4) \times 10^{-4}$ cm/s.

Similarly over the straight reach we find $u_e = (2.4 \pm 1.5) \times 10^{-3}$ cm/s. Hence the entrainment velocity is larger over the straight section by, typically, a factor of 16.

Both u_e and c are variable, but K_n and σ are constants in Long's model. Computing the mean square wave speeds separately for the sinuous and straight reaches of each of the four cruises we find $K_n\sigma^3 = u_ec^2 = 0.9 \pm 0.3$ and 6.4 ± 3.8 cm^3/s^3 on those two

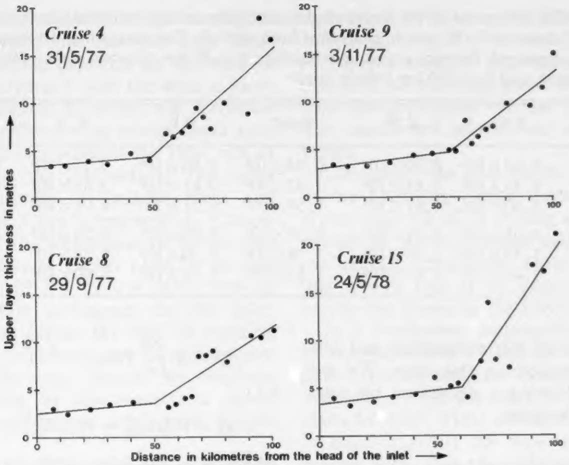


FIG. 4. Variation of upper layer thickness, determined by the objective method outlined in the text, versus distance (in kilometres) measured along the axis of Knight Inlet from the river input.

reaches, respectively. From the theoretical model we can easily derive:

$$K = \left(\frac{K}{mK_n}\right)\frac{K_n\sigma^3}{u_0 c_0^2}$$

and with the information above derive $K < 1.7 \times 10^{-3}$. It appears then that interfacial friction is not important in the circulation of Knight Inlet; we note in passing that Long's assumed value of $K = 1$ would lead to a friction dominated flow pattern. We obtain the best fit between observations and theory in the inviscid limit of $K = 0$. Gade and Svendsen (1978) find similar behavior and conclude that K is of order 10^{-3} for Sognefjord. Assuming an average value of $K_n\sigma^3 = 3.65$ and Long's value of $K_n = 0.1$, we find by substitution in Long's equation (40) that:

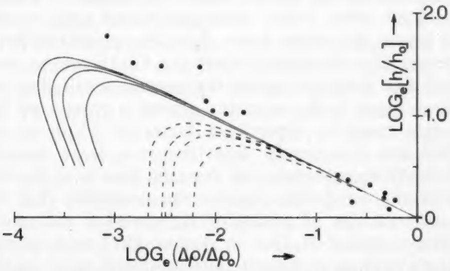


FIG. 5. The observed variation of $\Delta\rho/\Delta\rho_0$ with h/h_0. The points are taken from the observations listed in Table 1. The two families of curves drawn are the expected variation based on Long's model for the two values of the friction parameter K/mK_n; solid lines $K/mK_n = 0$, and broken lines $K/mK_n = 5$. Within each family the four curves correspond to each of the four cruises being considered, i.e. the variable parameter is initial Froude number F_0.

$$h_0 = 0.87q + 3.16 \times 10^5/q$$

where $q = (R/W)$ and R is the runoff and W the inlet width. Using values appropriate to cruises 4, 8, 9, and 15 we find h_0 to be 11.4, 10.7, 10.5, and 11.4 m, respectively. Furthermore, the smallest value that h_0 can take is 10.5 m. For those four cruises we observe h_0 to be 3.4, 2.5, 2.9, and 3.2 m, and so we clearly do not agree with Long's highly viscous, controlled model.

Finally, we would like to discuss the Froude numbers listed in Table 1. As mentioned earlier, the hydrography is not consistent with the condition $F = 1$ at the mouth. This is verified by the computations of F listed; however, it is striking just how far below critical the inlet appears to be. Apparently the inlet is not being controlled at the mouth. In Long's model the variation of h and u with distance along the inlet is determined by eliminating $(\rho_2 - \rho_1)$ from (5) using the Knudsen relations (2) and (3). This yields

$$dx = \frac{g(\rho_2 - \rho_0)}{\rho_0 K_n \sigma^3}\frac{d(hu_1)}{u_1}$$

which can be integrated numerically after the covariability of h and u is determined using (6a). This indicates that the inlet should be in the state $F_0 < F \leq \frac{1}{2}$ for most of its length and makes the final transition from $F = \frac{1}{2}$ to $F = 1$ in a comparatively short distance. However, implicit in this formulation is the proportionality of the entrainment velocity to the inverse Richardson number. Since $K_n\sigma^3$ is not a constant

TABLE 2. The divergence of the fluxes of potential, kinetic, and potential plus kinetic energy for the four cruises 4, 9, 15, and 8, clockwise from top left. The energy flux divergences are in J/s and are measured, for each cruise, over sections 0 to 50 km (sinuous reach), 50 to 100 km (straight reach), and 0 to 100 km (whole inlet).

	P.E.	K.E.	Total	P.E.	K.E.	Total
Sinuous	-0.04×10^6	0.10×10^4	-0.04×10^6	0.06×10^6	0.14×10^4	0.06×10^6
Straight	1.44×10^6	1.84×10^5	1.62×10^6	0.81×10^6	3.81×10^4	0.85×10^6
Whole	1.40×10^6	1.87×10^5	1.59×10^6	0.87×10^6	4.01×10^4	0.91×10^6
Sinuous	0.05×10^6	0.07×10^4	0.05×10^6	0.09×10^6	0.24×10^4	0.09×10^6
Straight	1.35×10^6	2.58×10^4	1.38×10^6	1.20×10^6	4.58×10^5	1.66×10^6
Whole	1.40×10^6	2.65×10^4	1.43×10^6	1.29×10^6	4.60×10^5	1.75×10^6

in the inlet we have to doubt this assumption and cannot apply the above equation in this case. We will return to this subject following a discussion on some energy considerations in the inlet.

Energy Considerations

Let us define $\chi(x)$ to be the anomaly of potential energy of the mean state at any point in the inlet, specifically χ is potential energy per unit surface area in a water column at position x minus that at $x = 0$.

Thus:

$$\chi(x) = g\int_{\text{bottom}}^{\text{top}} [\rho(x, z) - \rho(o, z)]z\,dz$$

$$= \tfrac{1}{2}g[(\rho_2 - \rho_1)h^2 - (\rho_2 - \rho_0)h_0^2]$$

for a two-layer fluid. If $P(x)$ is the rate at which energy is put into mixing in the fluid, per unit surface area, then we can write P as the divergence of the energy flux, i.e.

$$(11) \quad P(x) = \frac{d}{dx}[u_1(\chi + K)]$$

In (11) K is the kinetic energy contribution, $K = \tfrac{1}{2}\rho_1 u_1{}^2 h$. During one cruise a profiling current meter was used to examine the velocity structure at about km 70 in the inlet, averaged over a tidal cycle. It was interesting to note that the kinetic energy per unit surface area was an order of magnitude less than the potential energy anomaly; this will be verified shortly. The expression (11) does not include a contribution due to a change in the elevation of the free surface. We do not have a way of estimating this; however, one can show that in Long's model the ratio of potential energy change due to free surface changes and that due to internal changes is $\frac{1}{2}(\rho_2 - \rho_0)/\rho_0 < 0.04$, evaluated at the inlet mouth for the inviscid case. Hence, since Knight Inlet appears to belong to the inviscid regime we feel confident in ignoring the potential energy due to free surface modification.

Consider two reference sections which we will call A and B; then the total energy per unit time going into the mean state between those sections is

$$Q = W\int_B^A P\,dx,$$

i.e.

$$Q = W[u_1\chi]_B^A + W[u_1K]_B^A$$

where W is the inlet width. In Table 2 we list the energy input rates for each of the four cruises being considered. Listed separately are potential, kinetic, and total contributions for the sinuous, straight, and whole inlet segments. These energies are all derived from the data listed in Table 1. It is striking that the kinetic energy contributions are always substantially smaller than the potential energy contributions. This behavior is consistent with Long's model. What is perhaps more interesting, however, is that most of the energy put into the system is put in on the straight section. It is now clear why the entrainment velocity was found to be so much greater in the straight reach than in the sinuous reach. Some physical process that ultimately causes mixing in the fluid is considerably "more active" in the straight reach, and is doing more net work on the water columns of the straight reach.

We cannot explain the anomalous energy flux divergence on the sinuous reach for cruise 4. We have examined other cruise data and found only results similar to the other three data sets presented here. The negative divergence for cruise 4 is, however, very small and does not vitiate the general result that the energy input to the estuarine system is greater on the straight reach by typically a factor of 20. It is our belief that this results from the very large amount of turbulence generated by the tidal flow over the sill. However, we should consider the possibility that the wind is capable of producing the observed difference.

For a period of 23 d in August 1977 two anemometers in place in Knight Inlet produced good quality data simultaneously. One was about halfway along the sinuous section (near Tsukola Point, km 34) and the other was halfway along the straight section (km 74, near Hoeya Head). The rate at which wind does work on the sea surface is proportional to speed cubed (Kraus and Turner 1967). The ratio of mean-cube wind speed at the sill to that at Tsukola Point is 2.1 for the period in common. Since the data are dominated by energetic flows each afternoon, we assign one degree of freedom to each "event," i.e. one per day. Thus, we estimate 95% confidence intervals on the ratio to be ±0.5. Hence the difference is real; the wind is more intense on the straight section. However, the difference will not explain a 20:1 difference in energy input rate.

As a further check we can estimate the total rate of working by the wind on the sea surface. The cube-root–mean-cube wind speeds at Tsukola Point and Hoeya Head are 4.6 and 5.9 m/s, respectively. Choosing an average value for the whole inlet of 5.3 m/s we estimate, following Kraus and Turner (1967), the rate of working to be 3.7×10^5 J/s. This is about 25% of the observed energy flux divergence in the inlet. Furthermore, we do not expect the rate of working by the wind to produce turbulence and then mix water columns with 100% efficiency. Hence, we conclude that the rate of working by the wind is a minor contributor to the overall energy budget of Knight Inlet.

One of the more striking features of the oceanography of Knight Inlet is the presence of internal waves between Montagu Point and Siwash Bay. The waves are of very large amplitude, and on a quiet day can be heard. (Rapid convergence at the surface causes capillary waves to break causing a hissing sound.) The waves are described briefly by Pickard and Rogers (1959) and Pickard (1961). A detailed description of the waves is given by Farmer and Smith (1978), who show that the waves fit well into the theory of solitons described by Benjamin (1967). The mechanism by which they are generated has been observed and is described by Smith and Farmer (1977). There are, however, many subtleties associated with variations in the stratification and flow speed and these will be discussed in a later paper. For relatively weak stratification the generation mechanism appears to be similar to that described by Haury et al. (1979) for the generation of waves in the vicinity of Stellwagen Bank, and is roughly as follows:

i) The ebb tide flow over the sill develops and becomes as fast as a long interfacial wave; the internal Froude number becomes unity at the crest.

ii) The upper layer dives down to great depth on the lee side of the sill, as expected in a transition from sub- to super-critical flow, and then shallows abruptly via an internal hydraulic jump. Thus we have a large wave of depression on the lee side of the sill held stationary against an ebb tide flow.

iii) As the ebb tide decays the wave relaxes, broadens, propagates up the inlet, and disperses into a train of solitons.

This description appears not to work well during the summer months when the modal character of the response appears to change, though even then packets of highly nonlinear waves are formed. Though the mechanism is similar to that in the vicinity of Stellwagen Bank, the processes in Knight Inlet are very much more violent. A more detailed description of the variety of flows in the vicinity of the sill in Knight Inlet is given by Farmer and Smith (1980). In passing we should mention that the presence of these waves accounts for the large noise level in upper layer thickness determinations on the straight reach, an effect that would not affect near surface salinity measurements.

Ultimately, the sink for at least some of the energy in the hydraulic jumps, waves, and turbulence generated near the sill is mixing which raises the potential energy of water columns and can conceivably affect the general circulation. However, it is the source of this energy that is of immediate concern, and ultimately the source is the barotropic tide.

In a frictionless rectangular inlet the tide can best be represented as a standing wave. At each frequency, phase remains constant along the inlet except where a node occurs (if the inlet is very long) where phase shifts by 180°. Tidal elevations and tidal velocities are in quadrature. If the flow is made frictional simple models show that phase varies continuously along the inlet (Sverdrup et al. 1942, p. 574).

It is well known that the power dissipated in an inlet of arbitrary shape, upstream of any section is given by P, where:

$$P = \int_{\text{section area}} \rho\, g\, \overline{v\zeta}\, ds$$

where the overbar represents an average over a tidal cycle (Garrett 1975). Writing tidal evaluation $\zeta = \zeta_0 \sin(\omega t)$ and velocity $v = v_0 \cos(\omega t - \epsilon)$ we derive $\overline{v\zeta} = \frac{1}{2} v_0 \zeta_0 \sin(\epsilon)$. For a long, thin, rectangular inlet of length L_0, depth H, and width W in which only small phase changes occur, $v_0 \approx \zeta_0 L_0 \omega / H$ and we find:

$$P = \tfrac{1}{2} \rho g \zeta_0^2 \omega W L_0 \sin(\epsilon). \tag{12}$$

Current meters are noisy instruments, and it is hard to measure ϵ directly, but it is possible, in some circumstances, to estimate it from measurements of surface elevation alone.

Consider a model inlet as shown in Fig. 6. At two sections 1 and 2 tidal elevations are measured, and subscripts 1 and 2 will denote observations at those respective sections. Let us presume that all of the energy

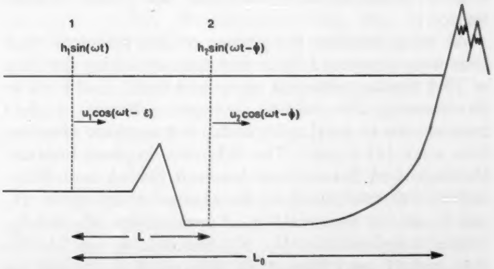


FIG. 6. Estuary model for tidal phase computations.

lost by a tidal wave is lost between sections 1 and 2. Hence, tidal elevations and velocities are in quadrature at section 2. However, energy is lost up inlet of section 1, so velocities and elevations are different in phase by $90° - \epsilon$, and elevations at 1 are different in phase from those at 2 by an angle ϕ. Applying volume conservation to the control region bounded by sections 1 and 2 we can relate ϵ and ϕ as follows:
the rate of accumulation of volume in the control region is

$$\begin{aligned}A_1u_1\cos(\omega t-\epsilon) - A_2u_2\cos(\omega t-\phi) = \\ \{A_1u_1\cos(\epsilon) - A_2u_2\cos(\phi)\}\cos(\omega t) + \\ \{A_1u_1\sin(\epsilon) - A_2u_2\sin(\phi)\}\sin(\omega t)\end{aligned}$$

where A and u represent inlet cross section and tidal velocity. We write the rate of increase of volume as the length of the section times the average width times the rate of increase of surface elevation. Hence, rate of volume increase is

$$\begin{aligned}\frac{L}{2}\left(W_1\frac{dh_1}{dt} + W_2\frac{dh_2}{dt}\right) = \\ \frac{\omega L}{2}[\{W_1h_1 + W_2h_2\cos(\phi)\}\cos(\omega t) \\ + W_2h_2\sin(\phi)\sin(\omega t)],\end{aligned}$$

where W is the width at the free surface.

Equating these two expressions and separating terms in $\cos(\omega t)$ and $\sin(\omega t)$ we derive:

$$\epsilon = \phi - \sin^{-1}(\mu\sin\phi) = (1-\mu)\phi \text{ if } \phi \text{ is small.} \tag{13}$$

$$\mu = \frac{\omega L W_1 h_1}{2A_1u_1} \approx \frac{L}{2L_0}.$$

Note that if L is much less than L_0, ϵ and ϕ are close to each other; however, if $L = L_0$, $\epsilon = \frac{1}{2}\phi$.

Tide gauges have been maintained at various sites in Knight Inlet since November 1976. The locations we will be concerned with are marked on Fig. 1 as M (Montagu Point), S (Siwash Bay), and W (Wahshihlas Bay). The sites were usually occupied by two independent gauges and the gauges were occasionally exchanged between different sites. This allowed us to identify timing problems and take appropriate measures. Generally there were no problems except for a small drift in the clocks of each instrument, which was easy to eliminate in estimates of the phase of tidal lines.

At each location the phases of the principal tidal lines were computed. This was done by taking the data in 29-d blocks, removing mean and trend, and Fourier transforming the residual. Successive blocks of data were chosen to overlap by 50%, so two phase observations were 14½ d apart. The difference in phase between Montagu and Siwash and between Siwash and Wahshihlas was computed at the diurnal components O_1 and K_1 and at the semidiurnal components M_2 and S_2. (Note: amplitudes at O_1, K_1 and M_2, S_2 are 25, 57, 160, and 51 cm.) The phase differences at the diurnal lines are small and probably not significant; however, the semidiurnal lines show large differences. Figures 7a and 7b show the phase differences at M_2 and S_2, respectively. The Montagu–Siwash differences are large and show large fluctuations, apparently seasonal; the Siwash–Wahshihlas differences are small and show small fluctuations.

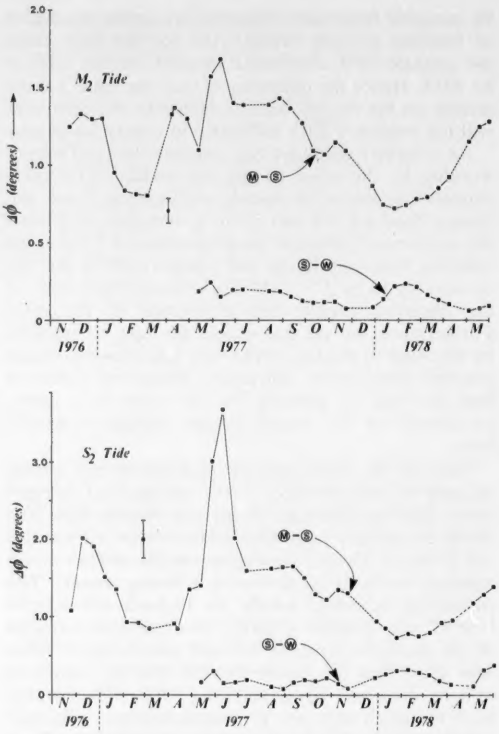


FIG. 7. Phase differences at tidal frequencies M_2 (7a, *upper* plot) and S_2 (7b, *lower* plot). In each diagram the upper curve shows the phase difference between Montagu and Siwash and the lower curve the phase difference between Siwash and Wahshihlas. Note: the station at Wahshihlas was installed later than the other two stations.

Confidence intervals can be estimated as follows: Halfway between the diurnal and the semidiurnal bands the root mean square (r.m.s.) noise level in tide gauge spectra in Knight Inlet is about 3 mm. If we imagine adding this noise level (a vector of amplitude N and random orientation) onto the M_2 vector, amplitude A, and given phase, we have a circle of uncertainty around the observation that subtends an angle of N/A radians. This would represent the error in phase in the worst possible case in which the noise and signal are orthogonal. The average error is easily seen to be $N/(A\sqrt{2})$ which translates into an expected error in phase determination of 0.08° and 0.24° for M_2 and S_2, re-

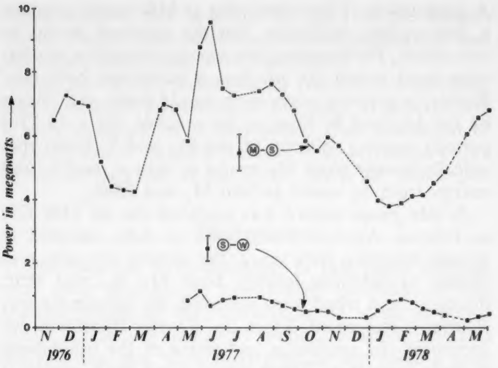


FIG. 8. Estimated power dissipation rates between Montagu and Siwash and between Siwash and Wahshihlas, *upper* and *lower* curves, respectively. Note: 1 MW = dissipation rate of 10^7 J/s.

spectively. Though this argument seems convincing to us, we believe that these confidence intervals are too large. If they really were this large we would expect a few of the M_2 and a large number of the S_2 phase differences between S and W to be negative. Though a negative difference would be unphysical (implying energy input to the tide from other inlet processes), random noise should produce some negative differences if the expected error is as large as we estimate. It is possible that cusping around the diurnal and semidiurnal bands has led to an inflated estimate of the noise level. The error bars derived above are entered on Fig. 7a and 7b and should be considered pessimistic estimates.

Using equations (12) and (13) we can convert the phase differences to estimates of energy dissipation rate, or power loss, from the tides between the observation points. In Fig. 8 is plotted the sum of the energy dissipation rates from M_2 and S_2 tidal lines. The upper and lower curves are for the regions between Montagu and Siwash and between Siwash and Wahshihlas, respectively. The application of these equations to the Siwash–Wahshihlas differences is correct and consistent with the approximations made earlier. Application to the Montagu–Siwash differences is not strictly admissible since there is some energy dissipation up-inlet of Siwash Bay. However, the change in phase up-inlet of Siwash is much less than that between Montagu and Siwash so the assumptions used to derive (12) and (13) may be considered a good approximation. It is clear from Fig. 8 that the energy dissipation rate on the straight reach far exceeds that on the sinuous reach. To determine how significant the difference is, we would like to determine the dissipation rate that would be expected from a well-documented source, bottom friction.

Consider a simple one-dimensional model of Knight Inlet in which we will allow the depth and width to vary. The momentum equation can be written:

$$\frac{\partial u}{\partial t} = -g\frac{\partial \zeta}{\partial x} - \frac{D(x)}{H(x)}.$$

Here, H is in the inlet depth, and $D(x)$ is a dissipation function; let us assume $D = ku|u|$. It is easily shown that if the total energy dissipation rate (averaged over one tidal cycle) is P, then

$$P = \int_{\text{mouth}} \rho g H \overline{u\zeta}\, d \quad = \int_{\text{surface}} \rho\, \overline{uD}\, dA.$$

{See Garrett (1975) for complete discussion.} Hence we can write

$$P = \rho k \int_{\text{surface}} \overline{|u|^3}\, dA.$$

If we assume that the free surface elevation behaves like $\zeta = \hat{\zeta} \sin(\omega t)$ and $\hat{\zeta}$ does not vary with x, then we can write a simple expression for u as

$$u = \frac{\omega \hat{\zeta} \xi}{H(x)} \cos(\omega t).$$

In the above ξ is a stretched x coordinate, specifically

$$\xi = \frac{1}{W(x)} \int_0^x W(x)\, dx,$$

and W is the inlet width. For an inlet in which W is close to being constant we can approximate ξ by x. We then derive

$$P = 0.4244\, \rho k \omega^3 \hat{\zeta}^3 \int_0^x \frac{W(x) x^3}{H^3(x)}\, dx$$

where $\overline{\cos^3(\omega t)} = 0.4244$. Observed values of k range between 0.001 and 0.004, according to Proudman (1953). Choosing a value of $k = 0.003$ we estimate the rate at which energy is lost up-inlet of Montagu Point to be 1.6×10^6 J/s. This is about 3% of the observed tidal energy loss rate. Clearly, bottom friction cannot account for the observed energy loss rate from the barotropic tides. The inlet is narrow, and so side friction may be important, but this is unlikely to double the energy loss rate.

The rate at which energy is lost in the sinuous reach, though fairly large, can be accounted for by bottom and side friction. We conclude then that processes peculiar to the straight reach, presumably internal wave and hydraulic jump generation, account for about 95% of the energy dissipation rate of the barotropic tide in Knight Inlet. The remaining 5% is lost to processes in common between the two regions, presumably bottom and side friction.

It is interesting to compare the energy flux divergences on the straight reach listed in Table 2 with the tidal energy dissipation rates calculated for the same reach. The latter are 8.85, 6.83, 5.84, and 6.6 MW for cruises 4, 8, 9, and 15, respectively. The energy flux divergences are then 1.8, 2.0, 1.5, and 2.5% of the tidal energy loss rate. If we assume that the tide is the dominant source of energy for mixing, then these percentages seem reasonable. We are postulating a multistep energy transfer (tidal energy loss converted to internal motions which lose energy into turbulence which mixes water columns) so we would not expect the total efficiency of energy exchange to be very high.

Let us return now to consideration of the circulation of Knight Inlet. We have seen that a certain amount of energy is required to drive the observed circulation, and a generous amount of energy is made available from the tides. In section 2 we claimed that we could not see the condition Froude number = 1 being achieved at the mouth. It is possible that the inlet could reach the state $F = 1$ in a very short distance beyond km 101 and still very close to the mouth, so let us calculate what additional energy input rate is needed to achieve that state for each of the four cruises we are considering here. If $F = 1$ at the mouth of the inlet then the total energy flux divergence for each of the cruises 4, 8, 9, and 15 would be 9.7, 5.1, 6.3, and 8.8×10^6 J/s. These energy flux divergences are typically six times the total observed between the inlet head and the station close to where we believe the mouth to be. It seems unlikely that energy can be put into the system as rapidly as seems to be required. We have observed spectacular mixing events close to the sill and yet this interaction produces only $\frac{1}{6}$ of the rate of input of energy required to drive the system with $F = 1$ at the mouth. We conclude that Knight Inlet is not "controlled" by the condition Froude number = 1 at the mouth.

Current Meter Observations

A mooring was deployed in Knight Inlet in January 1978, about 5 km seaward of the sill, and was recovered 3 mo later. The purpose of the mooring was to try to separate the baroclinic and barotropic tides and thereby obtain a direct estimate of the flux of tidal energy into the inlet. This was not successful, and the attempt will not be discussed here. However, the current meters (Aanderaa RCM-4s at depths of 15, 100, and 165 m) supplied another result that produced surprising confirmation of some of the notions expressed in this paper.

We have suggested that the dominant source of turbulence ultimately responsible for mixing in the inlet is energy extracted from the barotropic tide via internal wavelike processes at the sill. The hydraulic transitions that enable large amounts of energy to be put into internal motions do not occur on every tide, but occur on most ebb tides and some flood tides. In general the transitions prefer large tides, and so energy is more easily extracted at spring rather than neap tides. If more energy is made available for mixing at spring rather than neap tides, we expect the circulation to be modulated at the frequency of beating between M_2 and S_2 tides, i.e. at MSf, a period of 14.7 d. A modulation of the circulation at MSf would produce a free surface oscillation, but the response would be very small. Furthermore, the energy extraction mechanism itself would not produce a barotropic MSf tide. Rather, a periodic wave drag would cause the phases of the M_2 and S_2 lines to be variable (at a 14.77-d period) causing splitting of the M_2 and S_2 lines; specifically energy from M_2 would go into μ_2 and S_2 and energy from S_2 would go into M_2 and $2SM_2$.

A tide gauge record was analyzed for an MSf tide as follows. Approximately 3 mo of data, sampled at 15-min intervals, were taken; the number of points was chosen to minimize leakage from M_2, S_2, and MSf; the mean and trend were removed; the remainder was Fourier transformed. From the Fourier transform, we computed the amplitude and phase of the oscillations at each frequency. There was a small peak visible at MSf, the amplitude of the oscillation being 4.1 cm. If the entire surface of the inlet is moving up and down at this amplitude and with period 327.9 h, we expect a barotropic current at the current meter site of about 0.01 cm/s. The current meter records covered the same time period exactly and were treated in exactly the same way. However, one additional step was necessary: the current meters produce output of the E/W component of velocity and a N/S component, u and v, respectively. We computed the principal ellipse of fluctuations and rotated coordinate systems so that the u component contained the maximum fluctuation energy. We then examined the tidal content of this along-inlet flow. The starting time, total data length, and sampling interval were the same for the current meters and the tide gauge. The phase was corrected for drift in the clocks and the error due to different averaging intervals in the instruments.

The results for the MSf tide are shown in Table 3. The results shown for the tide gauge are the amplitude and phase estimated for the barotropic current based on the observed free-surface fluctuations. Clearly the currents observed far exceed the barotropic current expected. Furthermore, the deep currents are very close to being 180° out of phase with the near-surface currents indicating a purely internal response. The background noise level is fairly high at the 15-m current meter, about 1 cm/s, but drops rapidly at the

TABLE 3. Amplitude and phase of the MSf tide near Montagu Point, measured from the tide gauge and from current meters nearby. The amplitude actually seen at the tide gauge is converted to the barotropic velocity implied by that free surface motion, and the phase rotated by 90° to obtain the equivalent barotropic velocity signal at MSf.

Instrument	Amplitude (cm/s)	Phase
Tide gauge	0.01	−25.3°
Current meter at 15 m	4.85	−154.1°
" " " 100 m	1.14	11.9°
" " " 165 m	3.15	22.7°

deeper meters and is about 0.3 cm/s at the deepest meter. Hence, these data are observed sufficiently well above the noise level to be significant.

Analysis of the current meter records is continuing, and a detailed discussion of the tides in general will form the subject of a later paper. This discussion of the MSf tide is included because of its relevance to the processes outlined in the earlier parts of this paper.

Discussion

The data we have discussed in this paper indicate that there are several outstanding problems in our understanding of the circulation of deep stratified inlets. According to Long's model (which synthesizes many ideas that have been popular for quite a long time), the depth of the interface at the head of the inlet is determined by the river runoff, the amount of turbulence available for mixing, and the existence of a hydraulic control at the mouth. We claim that the flow is not in fact controlled at the mouth, at least not by the condition $F = 1$. There are three main reasons: the hydrography implied does not occur; the calculated speeds are far too low; and there is insufficient energy available, by a wide margin, to drive the inlet to the condition $F = 1$ from the observed conditions at the head. Otherwise the circulation is fairly well described by Long's model, providing that a small friction coefficient is chosen. This state of affairs is unsatisfactory. If we reject the notion of control at the mouth, then we have to ask, why was the interface specifically 3.38 m deep at the head on May 31st, 1977? Then we must ask, why did it change at other times? We do not have answers to these questions.

We have presented evidence in this paper to the effect that internal hydraulic transitions are crucial to the energy-exchange processes in Knight Inlet. The energy source is the barotropic tide, the sink is divergence of mean potential energy flux, and the waves may form an intermediate stage useful for distributing energy in the inlet. Several disparate observations indicate that this process is dominant and we are able to compute an efficiency for the overall exchange of energy. However, to what extent is this process peculiar to Knight Inlet? In short, we honestly do not know. There is, however, some evidence that the processes operating in Knight Inlet may occur elsewhere. Pickard (1961) discusses the internal waves in Knight Inlet at some length and reports that waves have been observed in Bute Inlet, though these appear to be somewhat different from those in Knight. He also reports waves in Burke and Observatory inlets. There are indications that those in Observatory Inlet are of very large amplitude. Similar waves are a common feature of some other parts of the British Columbia coastline, e.g. Georgia Strait. We conclude then that the mechanisms outlined here may not be peculiar to Knight Inlet, and indeed may constitute an efficient driving mechanism for a particular class of inlets.

Finally we comment on an approximation made by Long (1975). In deriving the upper layer momentum equation he neglects a term in his equation (8), representing the exchange of momentum between the upper and lower layers by the entrainment velocity. The term is neglected on the basis that it is much smaller than the interfacial friction term. Since we find interfacial friction to be very small we conclude that this term should be retained. Retaining the term alters the left hand side of equation (1) to:

$$\frac{d(u_1{}^2h)}{dx} - u_e u_i.$$

Here, u_e is the entrainment velocity and u_i is the horizontal velocity at the base of the upper level. Let us set $u_i = \epsilon u_1$ and proceed. The resulting set of equations is easily solved, and we derive an equivalent of equation (6a) as

$$\left(\frac{h}{h_0}\right) = \frac{u_1}{u_0}\left[\frac{2(1-\epsilon+\mu)\,u_0{}^3 + u_0 c_0{}^2}{2(1-\epsilon+\mu)\,u_1{}^3 + u_0 c_0{}^2}\right]^q$$

where $q = \tfrac{1}{3}(3 - 2\epsilon + 2\mu)/(1 - \epsilon + \mu)$ and $\mu = K/mK_n$.

For reasonable values of ϵ (presumably $0 < \epsilon < 1$) we find that the overall behavior is close to that in Long's model. For low velocities, where the nonlinear terms are not important anyway, the two solutions are indistinguishable. For any $\epsilon > 0$ the greatest value of h possible is greater than that for $\epsilon = 0$. Furthermore, the Froude number at which the maximum value of h occurs is displaced to higher values. In the special case of inviscid flow and $\epsilon = 1$ we derive

$$\ln(h/h_0) = \ln(u_1/u_0) - \tfrac{2}{3}(u_1{}^3 - u_0{}^3)/(u_0 c_0{}^2).$$

In this case the maximum value of h occurs at Froude number $1/\sqrt{2}$ rather than $1/2$ when $\epsilon = 0$. Otherwise the behavior is hardly changed and the conclusions reached in this paper remain unchanged by the retention of this term.

Acknowledgments

The Knight Inlet project was a large effort requiring dedicated work by many individuals. We thank the technicians and scientists who participated in the many cruises involved and all who assisted with data processing. We also would like to acknowledge the assistance and advice of Dr R. R. Long; his comments have added greatly to the work reported on here.

BENJAMIN, T. B. 1967. Internal waves of permanent form in fluids of great depth. J. Fluid Mech. 29: 559–592.

FARMER, D. M., AND J. D. SMITH. 1978. Nonlinear internal waves in a fjord, p. 465–493. *In* J. Nihoul [ed.] Hydrodynamics of estuaries and fjords, Elsevier, Amsterdam.

1980. Stratified flow over a sill in Knight Inlet, British Columbia. Deep-Sea Res. 27: 239–254.

GADE, H. G., AND E. SVENDSEN. 1978. Properties of the Robert R. Long model of estuarine circulation in fjords,

p. 423–437. *In* J. Nihoul [ed.] Hydrodynamics of estuaries and fjords, Elsevier, Amsterdam.

Garrett, C. J. R. 1975. Tides in gulfs. Deep-Sea Res. 22(1): 23–36.

Haury, L. R., M. G. Briscoe, and M. H. Orr. 1979. Tidally generated internal wave packets in Massachusetts Bay. Nature 278: 312–317.

Kantha, L. H. 1975. Turbulent entrainment at the density interface of a two-layer stably stratified fluid system. Ph.D. thesis, The Johns Hopkins University, Baltimore, MD, USA.

Kato, H., and O. M. Phillips. 1969. On the penetration of a turbulent layer into a stratified fluid. J. Fluid Mech. 37: 643–655.

Kraus, E. B., and J. S. Turner. 1967. A one-dimensional model of the seasonal thermocline. II. The general theory and its consequences. Tellus XIX: 98–106.

Long, R. R. 1975. Circulations and density distributions in a deep, strongly stratified, two-layer estuary. J. Fluid Mech. 71: 529–540.

Pearson, C. E., and D. F. Winter. 1978. Two-layer analysis of steady circulation in stratified fjords, p. 495–514. *In* J. Nihoul [ed.] Hydrodynamics of estuaries and fjords. Elsevier, Amsterdam.

Pedersen, Fl. Bo. 1978. A brief review of present theories of fjord dynamics, p. 407–422. *In* J. Nihoul [ed.] Hydrodynamics of estuaries and fjords. Elsevier, Amsterdam.

Pickard, G. L. 1961. Oceanographic features of inlets in the British Columbia coast. J. Fish. Res. Board Can. 18: 907–999.

Pickard, G. L., and K. Rodgers. 1959. Current measurements in Knight Inlet, British Columbia. J. Fish. Res. Board Can. 16: 635–678.

Proudman, J. 1953. Dynamical Oceanography. Methuen, London/Wiley, New York, N.Y. 409 p.

Smith, J. D., and D. M. Farmer. 1977. Nonlinear internal waves and internal hydraulic jumps in a fjord. *In* Geofluiddynamical wave mathematics. Applied Mathematics Group, University of Washington, Seattle, WA.

Stommel, H. M., and H. G. Farmer. 1953. Control of salinity in an estuary by a transition. J. Mar. Res. 12(1): 13–20.

Sverdrup, H. U., M. W. Johnson, and R. H. Fleming. 1942. The Oceans: their physics, chemistry and general biology. Prentice-Hall, Englewood Cliffs, NJ. 1087 p.

Resource Partitioning in Two Coexisting Sunfish: Pumpkinseed (*Lepomis gibbosus*) and Northern Longear Sunfish (*Lepomis megalotis peltastes*)

DENNIS R. LAUGHLIN AND EARL E. WERNER

W. K. Kellogg Biological Station and Department of Zoology, Michigan State University, Hickory Corners, MI 49060, USA

LAUGHLIN, D. R., AND E. E. WERNER. 1980. Resource partitioning in two coexisting sunfish: pumpkinseed (*Lepomis gibbosus*) and northern longear sunfish (*Lepomis megalotis peltastes*). Can. J. Fish. Aquat. Sci. 37: 1411–1420.

Qualitative surveys of 22 lakes in southern Michigan indicated that relative abundances of the northern longear sunfish (*Lepomis megalotis peltastes*) and pumpkinseed (*L. gibbosus*) depended upon amount of plant cover and sediment type. The longear sunfish occurred only in lakes with marl sediments and regions of sparse vegetation cover. The pumpkinseed occurred in all lakes surveyed and increased in abundance in lakes with greater vegetation density and/or more organic sediments. Quantitative measurements of habitat and food use were made in five lakes with sympatric populations. Data from underwater transects indicated that large (>75 mm standard length) longear sunfish occurred primarily in shallow regions containing moderate to low vegetation cover whereas large pumpkinseeds were concentrated in deeper areas with heavy vegetation cover. The smaller size-classes of both species occupied the vegetation in deeper areas of the littoral zone. Large longears consumed predominantly large, sediment-dwelling mayfly or odonate nymphs regardless of the lake or habitat types. Pumpkinseeds consumed mainly gastropods and other prey from the vegetation. Preliminary studies of seasonal food use indicated that the pumpkinseed and longear sunfish both consumed more food in the spring and exhibited greater overlap in diet during this period. The relationship between foraging behaviors of these two species and the types of prey consumed is discussed as well as the complications of assessing competition between species when food and habitat use are dependent on size.

Key words: Habitat use, size-class, lake types, food overlap, competition, *Lepomis*, Michigan

LAUGHLIN, D. R., AND E. E. WERNER. 1980. Resource partitioning in two coexisting sunfish: pumpkinseed (*Lepomis gibbosus*) and northern longear sunfish (*Lepomis megalotis peltastes*). Can. J. Fish. Aquat. Sci. 37: 1411–1420.

On constate, après relevés qualitatifs de 22 lacs dans le Michigan méridional, que l'abondance relative des crapets à longues oreilles (*Lepomis megalotis peltastes*) et des crapets-soleils (*L. gibbosus*) dépend de la quantité de couverture végétale et du type de sédiment. Le crapet à longues oreilles ne se rencontre que dans les lacs à sédiment de marne et couverture végétale clairsemée. Le crapet-soleil se trouve dans tous les lacs étudiés et est plus abondant dans les lacs à végétation plus dense et/ou sédiment organique plus abondant. Des mesures quantitatives d'habitat et d'utilisation de la nourriture ont été effectuées dans cinq lacs contenant des populations sympatriques. Des observations le long de sections sous l'eau indiquent que les grands crapets à longues oreilles (>75 mm de longueur standard) se trouvent surtout dans les régions peu profondes, à couverture végétale allant de modérée à faible, alors que les grands crapets-soleils sont concentrés dans les régions plus profondes, à dense couverture végétale. Les petites classes de taille des deux espèces se tiennent parmi la végétation dans les régions profondes de la zone littorale. Les crapets à longues oreilles consomment surtout des éphémères habitant le sédiment ou des nymphes d'odonates, quel que soit le type de lac ou d'habitat. Les crapets-soleils consomment surtout des gastéropodes et autres proies extraites de la végétation. Des études préliminaires sur l'utilisation saisonnière de la nourriture indiquent que les crapets-soleils et les crapets à longues oreilles consomment, les uns et les autres, plus de nourriture au printemps, époque à laquelle il y a plus de chevauchement des régimes. Les auteurs analysent la relation entre les moeurs fourragères des deux espèces et les types de proies consommées, de même que les problèmes que soulève l'évaluation de la consurrence interspécifique quand l'utilisation de la nourriture et de l'habitat dépend de la taille.

Received January 3, 1980
Accepted May 22, 1980

Reçu le 3 janvier 1980
Accepté le 22 mai 1980

Printed in Canada (J5912)
Imprimé au Canada (J5912)

STUDIES of resource partitioning in freshwater fish communities (Keast 1978; Werner et al. 1977) are relatively rare compared to such studies in other groups (Schoener 1974). Further, these studies generally do not evaluate food and habitat use concurrently, which hinders the interpretation of changes in niche overlap over time or space. Such studies, however, are a necessary prelude to more detailed work on the mechanisms responsible for ecological segregation (Werner and Hall 1977, 1979) and suggest patterns in the structure of fish communities. In addition, fish populations are strongly size-distributed and provide an excellent opportunity to examine the effects of size on food and habitat use and the implications to species segregation.

This study focuses on the food and habitat use of two closely related sunfishes, the northern longear sunfish (*Lepomis megalotis peltastes*), and the pumpkinseed (*L. gibbosus*). These species commonly coexist in the small (<20 ha) soft-bottomed lakes of southern Michigan and are morphologically very similar (Trautman 1957). The northern longear sunfish is a dwarfed subspecies of the central longear (*L. m. megalotis*) and may well be a distinct species (Trautman 1957). The northern longear's range extends from Lake Ontario through the lower Great Lakes west to Iowa. The pumpkinseed sunfish is widely distributed throughout the northern half of eastern North America, from the Carolinas north to southern Ontario.

In small southern Michigan lakes both species are found in the littoral zone and are closely associated with the sediments (Werner et al. 1977). Published accounts of the pumpkinseed's diet indicate that it uses considerable amounts of benthic invertebrates and molluscs (Ball 1948; Keast 1978; Seaburg and Moyle 1964). While the northern longear's behavior has been extensively studied (Smith 1971; Keenleyside 1967), we know of no published accounts of its ecology. The behavioral and morphological similarities of these two species and their close association prompted a detailed study of their ecological relations.

The objectives of this study were to determine if presence or abundance of these species was associated with macrohabitat characteristics and to examine the coutilization of resources when both species occurred in the same lake. The approach adopted was comparative in nature. A broad qualitative survey of small lakes was undertaken to examine the status of these species in relation to macrohabitat, and a more intensive study of food and habitat use was performed in five lakes where both species coexisted.

Methods

Qualitative surveys were conducted in 22 lakes in Barry or Kalamazoo counties, Michigan, by swimming the littoral zones with mask and snorkel and making rough counts of each species. Vegetation type and density and sediment type (marl, organic, etc.) were likewise noted. Five lakes (Deep, Shaw, Bassett, Hamilton, and Lawrence) were then chosen for more intensive study; criteria were that lake morphometry and water clarity permitted accurate visual censusing, and, that a range of distinct habitats (both within and between lakes) was available for examining resource use of the two species. Each of these lakes contained both fish species. Detailed descriptions of the study lakes can be found in Laughlin (1979).

The census methods used were similar to those of Werner et al. (1977). Transects parallel to the shoreline were established at various measured distances from shore. A single observer using snorkel or SCUBA gear swam each transect in succession, starting with the nearshore transect and finishing with the transect at the limits of rooted aquatic vegetation (4- to 5-m depth). These transects provided censuses of representative habitat types in the littoral zone and ranged from 200 to 600 m in length. Previous investigations had indicated that longears were closely associated with the nearshore areas of lakes, so the two transects nearest the shore were closer together (1.5–3 m apart) to document fully any changes in longear numbers with distance from shore. The remaining transects were chosen to include depth zones which would characterize the changes in habitat structure with depth.

Each longear and pumpkinseed encountered was recorded on premarked sheets of underwater paper (Ascot) according to species and size. The size-categories were small (<38 mm total length), medium (39–75 mm), large (76–125 mm), and extra large (>125 mm). Censuses were replicated at least 4 times during the summer in each lake and were performed seasonally in Deep Lake.

Habitat measurements were made on each of these transects in August 1977. At intervals determined by 30 strokes of the observer's flippers, a 0.09-m^2 area was censused by blindly placing a grid on the lake bottom (10 to 30 samples per transect). The following measurements were taken: (1) the number (out of 25) of grid intersections underlaid by vegetation (as an index of percent cover), (2) the actual numbers of each plant species or a count of a recognizable structure of the species when individual plants were not distinguishable (e.g. *Chara*, *Najas*, *Elodea*), and (3) the average height of the vegetation from the sediments.

Fish of both species were collected for analysis of stomach contents from specific habitats. Midsummer daytime collections were made in all study lakes except Lawrence Lake; seasonal trends of food use were investigated in Deep and Hamilton Lakes. Longears were easily collected by a diver with a long-handled net since they remained sedentary and close to the bottom even around an observer. Pumpkinseeds were more difficult to capture, and required the use of a seine, spear gun, or hand net. Once captured, fish were killed in the anesthetic MS-222 and preserved in 10% Formalin in the field.

Fish were sexed and measured (standard length) in the laboratory. Stomach contents were identified to the lowest taxonomic level possible, and prey body length, head width, or some other feature measured and an appropriate regression used to calculate dry weight. Regressions were determined directly or were obtained from the literature. Each prey type was placed in one of three habitat associations according to Laughlin (1979): (1) sediment, (2) vegetation, or (3) "other" (when no clear assignment was possible). Habitat association of the prey was determined by natural history accounts of the organisms (Pennak 1954; Merritt and Cummins 1978; Corbet 1954).

TABLE 1. Qualitative surveys of the relative abundances of northern longear sunfish (*Lepomis megalotis peltastes*) and pumpkinseed (*L. gibbosus*) from 22 southern Michigan lakes. All lakes are located in Barry or Kalamazoo counties, Michigan. Dominant sediment type and relative vegetation density in the littoral zone are also noted.

Lake	Dominant sediment type	Vegetation density	Abundance	
			Longear	Pumpkinseed
Bassett	Marl	Light	Abundant	Moderate
Deep	"	Moderate	Abundant	Moderate
Hamilton	"	Heavy	Moderate	Abundant
Lawrence	"	Moderate	"	Moderate
Tamarack	"	"	"	"
Shaw	"	"	"	"
Pine	"	"	"	"
Palmatier	"	Heavy	Rare	Abundant
Turner	Organic	Heavy	Rare	Abundant
Long	Marl	Light	Absent	Moderate
Little Mill	"	Heavy	"	Moderate
MacDonald	"	Light	"	Moderate
Three	"	Heavy	"	Abundant
Hall	Organic	Moderate	"	Moderate
Warner	"	Moderate	"	Abundant
Williams	"	Heavy	"	Moderate
Chief Noonday	"	Moderate	"	"
Baker	"	Moderate	"	"
Culver	"	Heavy	"	"
Stewart	"	"	"	"
Cassidy	"	"	"	"
Podunk	"	"	"	"

Stomach analyses indicated that different prey sizes were used by these two species. Experiments were therefore conducted to determine if these species differed in their abilities to handle different prey sizes. Individual fish were fed free-swimming *Hexagenia* (a common food item) one at a time in laboratory aquaria. The time required to handle and swallow each item was timed with a stopwatch. Each fish was fed several items per trial and then fed a constant amount of dry food afterwards.

Results

The 22 lakes surveyed are listed in Table 1, along with qualitative descriptions of species abundances, dominant sediment types, and vegetation densities. The pumpkinseed occurred in every lake that was surveyed; the longear occurred only in lakes containing areas of exposed marl sediments and were generally absent or rare in lakes with dense stands of vegetation covering the shallow (<2 m) littoral zone, regardless of sediment type. The longear was absent in a few apparently suitable lakes (e.g. MacDonald, Long).

Deep and Turner lakes, separated only by a 0.4-km creek, clearly showed the contrasting distributions of the longear and pumpkinseed. Deep Lake (sediment organic matter = 7%) supported a very abundant longear population, whereas longears were essentially absent from Turner Lake (sediment organic matter = 37%). Pumpkinseeds occurred in both lakes. Deep Lake contained shallow areas grading from sparse to heavy vegetation whereas the shallow expanses of Turner were covered with dense growths of *Myriophyllum*, *Nuphar*, *Elodea*, and *Chara*.

HABITAT UTILIZATION

A comparison of habitat use patterns showed that species distributions for similar habitats across lakes were much more alike than areas of different habitat structure within lakes. Consequently, in the following analyses we combine the data for similar habitats from different lakes and compare fish distributions on a habitat specific basis. We consider first the distributions in midsummer which are generally stable from June through August (Hall and Werner 1977), and then examine the seasonal patterns in Deep Lake.

Regions with open marl bench — Three of the study lakes (Shaw, Lawrence, and Deep) had shallow marl benches extending ~2 to 12 m from shore which dropped off sharply in the slope region. These benches were sparsely vegetated nearshore with cover increasing markedly further offshore; dense stands of *Chara* or *Scirpus subterminalis* occurred on the slopes.

The large longears and pumpkinseeds were clearly segregated; large longears were concentrated at the nearshore position in all three lakes (Fig. 1). Large pumpkinseeds on the other hand exhibited a closer association with offshore vegetation (Fig. 1), though

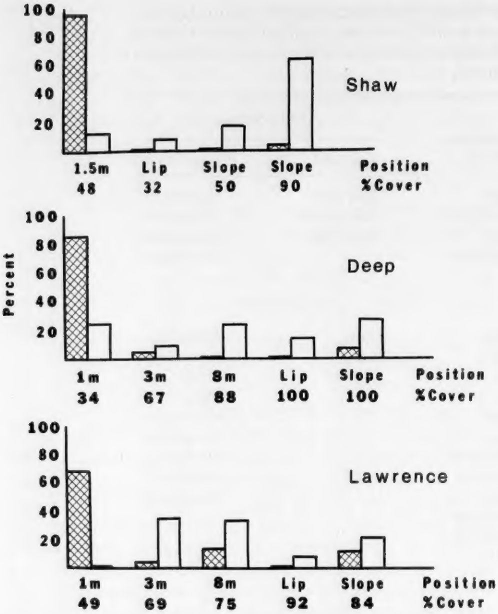


FIG. 1. Average proportional abundance of longear (cross-hatched histograms) and pumpkinseed (open histograms) sunfish greater than 75 mm in length at different distances from shore in three different lakes. Average percent cover by vegetation is indicated under each position. Numbers on the abscissa are distances from shore on the bench; the lip represents the beginning of the steep slope region and is about 1–2.5 m deep. Slope transects were at the 3- to 4-m depth contour except in Shaw Lake where two slope transects were censused, one at 2.5 m and the other at 4.5 m.

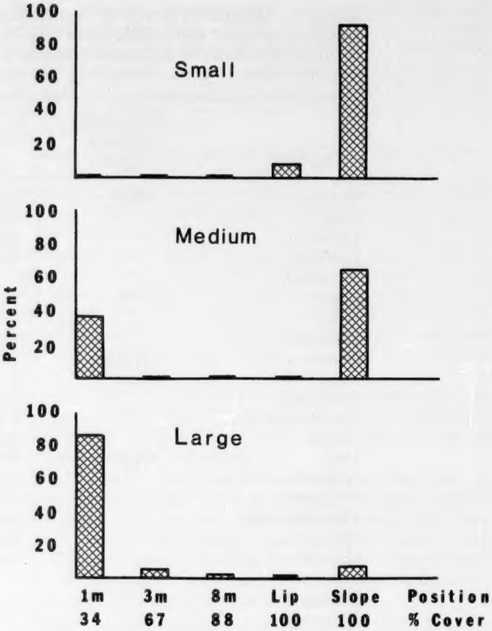


FIG. 2. Average proportional abundance of the three size-classes of longear sunfish at different distances from shore in a region of Deep Lake that had a sparsely vegetated bench. Average percent cover by vegetation is indicated under each position. Positions are as defined in Fig. 1.

large pumpkinseeds did occur over bare marl sediments in Lawrence Lake.

The smaller size-classes of both species exhibited dramatically different distributions. In both Deep and Lawrence lakes, medium longears were evenly divided between the nearshore position and heavier vegetation on the slope whereas small longears occurred almost exclusively in deep water stands of *Myriophyllum*, *Scirpus*, *Potomogeton*, and *Chara* (Fig. 2). Small and medium pumpkinseeds were even more concentrated in areas of heavy vegetation than the small longears (Fig. 3). As a consequence the overlap in habitat between small size-classes of these two species was much greater than for the large size-classes (Fig. 1 and 3).

A concurrent separation by sex was related to the distribution of longear size-classes. The number of males and females collected for food analysis nearshore in Deep Lake versus those collected on the slope are as follows.

	Nearshore	Slope
Males	63	59
Females	29	71

The mean fish size was 80.1 ± 1.5 mm nearshore and 43.9 ± 1.7 mm on the slope. The proportions of males and females differed significantly in the two areas ($P < 0.0005$, *G*-test, Sokal and Rohlf 1969). Sexual segregation here is probably the result of the size differences between male and female longears since male longears have a higher growth rate than females (Hubbs and Cooper 1935).

Regions with vegetated marl bench — Certain changes in distribution of the fish were noted in two of the study lakes (Deep and Hamilton) that contained sites with densely vegetated bench regions (primarily *Chara*). This region in Deep Lake contained only ⅓–½ as many longears as the open bench region, the reduction being primarily in the largest size-class. Longears were more evenly distributed over densely vegetated benches than open benches (Fig. 4). The larger pumpkinseeds were also broadly distributed over the vegetated benches (Fig. 4). The smaller pumpkinseeds and longears showed little change in habitat use from the previous study sites.

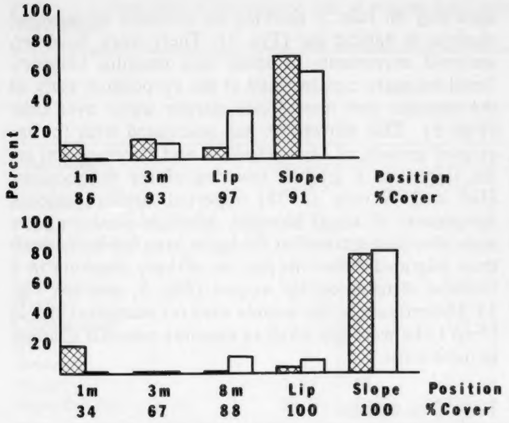


FIG. 3. Average proportional abundance of longear (cross-hatched histograms) and pumpkinseed (open histograms) sunfish less than 75 mm in length at different distances from shore in Deep Lake. The data in the top panel are from a region with a densely vegetated bench and in the bottom panel from a region with a sparsely vegetated bench. Average percent cover by vegetation is indicated under each position. The longear sunfish at the 1- and 3-m positions are all from the 39- to 75-mm class (medium) which shows a bimodal distribution (Fig. 2). Positions are as defined in Fig. 1.

Finally, Bassett Lake differed from all others in that submersed plant cover was very sparse and concentrated at the lip and shallow parts of the slope, the shoreline lacked emergent vegetation, and marl sediments were replaced by sand nearshore. There was a striking shift of all size-classes of both species to the vegetation at the lip and shallow slope. Over 93% of all individuals of both species were found in a strip 1–2 m wide in this region. Pumpkinseeds were also much less abundant overall than the longear (2.4 vs. 18.5/100 m).

HABITAT USE AND VEGETATION STRUCTURE

A partial correlation analysis relating fish abundance to mean height of vegetation and mean number of grid points covered by vegetation (a measure of percent cover) was performed using data from two census dates (July 15 and August 1, 1977) in Deep Lake in conjunction with the set of habitat measurements taken shortly thereafter. Smaller size-classes of both species were positively associated with vegetation height (Table 2). Large longears were negatively associated with vegetation height. Further, densities of large and medium longears were negatively correlated with percent cover. The remainder of the partial correlations factoring out the effects of percent cover were nonsignificant.

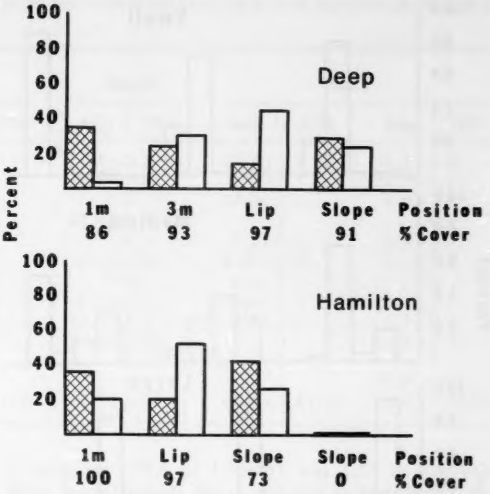


FIG. 4. Average proportional abundance of longear (cross-hatched histograms) and pumpkinseed (open histograms) sunfish greater than 75 mm in length at different distances from shore in two lakes with densely vegetated bench regions. Average percent cover by vegetation is indicated under each position. Positions are as defined in Fig. 1. The two slope positions in Hamilton Lake were at the 3- and 5-m depth contours.

Despite the negative correlation with percent cover, very few large longears used areas of barren marl with <10% plant cover. The highest density of large longears in Deep Lake was associated with a mean percent cover of 39%. Even heavily vegetated areas were used more extensively than barren marl, resulting in a habitat utilization curve on a percent cover axis that is very thin-tailed at low percent cover values and relatively thick-tailed at higher values.

TABLE 2. Partial correlation analysis of longear and pumpkinseed abundance with mean percent cover and mean vegetation height in Deep Lake (1 = mean percent cover, 2 = mean vegetation height, 3 = number of fish per metre of transect). Small pumpkinseeds were not censused in sufficient numbers to include in the analysis.

		Partial correlation coefficients	
Fish species	Fish size	Percent cover $r_{31\cdot2}$	Height $r_{32\cdot1}$
Longear	Small	−0.43	0.85**
	Medium	−0.62*	0.51
	Large	−0.82**	−0.67**
Pumpkinseed	Medium	−0.34	0.74**
	Large	0.10	−0.30
	Extra large	0.06	−0.24

*$P < 0.05$.
**$P < 0.01$.

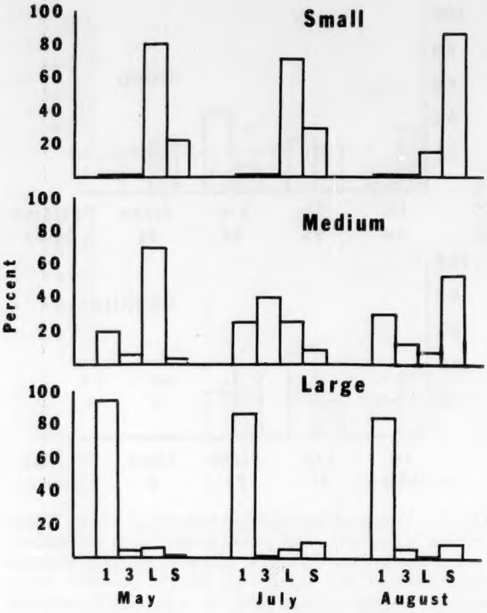


FIG. 5. Average proportional abundance of the three size-classes of longear sunfish at different distances from shore in Deep Lake in May, July, and August. The 8-m position contained very few fish and was omitted. Positions are as described in Fig. 1.

SEASONAL TRENDS IN HABITAT USE

Seasonal patterns in habitat use of the longear were examined in Deep Lake from May through August. Longear sunfish appeared in the littoral zone nearshore during the 1st wk of May. The large longears occurred almost exclusively at the nearshore position both during the month of May and immediately after spawning on July 5, showing no evidence of seasonal changes in habitat use (Fig. 5). There were, however, seasonal movements of small and medium longears. Small longears concentrated at the lip position early in the summer and moved into deeper water over time (Fig. 5). This movement was associated with the increased growth of *Myriophyllum* and *Potomogeton* on the slope and a gradual lowering of the thermocline. Hall and Werner (1977) reported similar seasonal movements of small bluegills. Medium-sized longears were also concentrated at the lip in May but individuals then migrated either inshore or offshore resulting in a bimodal distribution by August (Fig. 5, also see Fig. 2). Unfortunately, the sample sizes for pumpkinseeds in Deep Lake were too small to examine seasonal changes in habitat use.

FOOD UTILIZATION

The diets of the longear and pumpkinseed provide detail on microhabitat use that complements the data on macrohabitat distribution. Larger longears foraged principally from the sediments regardless of vegetation density whereas pumpkinseeds utilized more vegetation prey.

Large longears were collected in 1976 and 1977 in Deep, Hamilton, and Shaw lakes and only in 1977 in Bassett Lake. The diets were remarkably similar across years and lakes despite differences in vegetation density and distribution. Large longears contained primarily aeschnid, libellulid, and gomphid dragonfly nymphs, *Caenis* nymphs, the amphipod *Hyallela azteca*, and the mayfly *Hexagenia limbata* (Tables 3, 4). Most of the prey were classified as sediment-dwelling, averaging about 54% of the diet by weight. Large aeschnid dragonfly nymphs comprised the bulk of the vegetation prey (particularly in 1976 in Shaw Lake).

Pumpkinseeds were collected in 1976 and 1977 in Hamilton Lake and 1977 in Shaw, Deep, and Bassett lakes. Pumpkinseeds collected in Shaw and Hamilton contained predominantly gastropods (Table 5), which

TABLE 3. Mean percent dry weight (±SE) of selected food items in the stomachs of longear sunfish from Deep Lake.

	May 14–24, 1977	July 5, 1977	Aug. 7–13, 1977	Aug. 10, 1976	Aug. 18, 1976
Libellulids	12.3±4.5	13.4±7.3	17.5±1.8	21.1±6.1	22.3±8.9
Gomphids	20.7±6.1	37.8±9.7	16.5±3.5	15.5±8.9	30.0±7.2
Aeschnids	0.2±0.2	11.1±5.8	21.9±1.6	7.9±5.3	6.9±3.6
Hexagenia	0	0	0.4±0.3	4.2±3.8	0
Coenagrionids	21.3±3.2	4.4±1.8	1.5±0.5	2.2±1.2	6.3±3.2
Caenis	8.2±2.1	6.0±2.5	8.3±1.9	9.0±5.2	0.2±0.1
Ephemerella	19.5±4.8	0	0	0	0
Hyallela	6.8±1.6	13.2±4.6	20.2±3.4	15.6±6.4	3.8±1.8
Chironomids	2.3±0.9	4.2±1.8	2.1±1.2	2.9±1.3	1.2±0.4
Heliosoma	0	0	0.05±0.05	0	0
Amnicola	0	0	0	0.06±0.05	0
Physa	0	0	0	1.0±1.0	0
Mean fish size ± SE (*n*)	80.4±2.0 (25)	79.3±2.1 (16)	79.9±1.3 (47)	80.7±1.9 (13)	71.2±4.2 (11)

TABLE 4. Mean percent dry weight (±SE) of selected food items in the stomachs of longear sunfish from Hamilton, Bassett, and Shaw Lakes.

	Hamilton		Bassett	Shaw	
	May 8–12, 1977	Aug, 15, 1976	Aug. 9, 1976	Aug. 21, 1976	Aug. 5, 1977
Libellulids	0.05±0.05	11.4±6.5	3.5±2.1	17.2±6.9	12.9±4.0
Gomphids	12.1±4.7	33.3±8.7	10.5±3.2	5.8±5.3	29.7±6.7
Aeschnids	0	0	0	22.8±9.1	6.9±3.8
Hexagenia	4.7±3.3	14.4±6.4	47.9±5.6	0	0
Coenagrionids	26.9±5.5	0.4±0.4	0	0.2±0.2	0.4±0.4
Caenis	6.4±1.8	0	0.6±0.4	0.03±0.03	5.3±2.4
Ephemerella	31.6±5.8	2.5±2.0	0	1.6±1.1	0
Hyallela	3.7±2.1	3.1±3.1	5.7±1.4	4.8±2.5	15.4±2.8
Chironomids	1.1±0.3	3.4±2.1	14.4±3.2	8.4±4.6	1.7±0.4
Helisoma	0	0	0.02±0.02	0	0.18±0.18
Amnicola	0	0	0	0	0
Physa	0.25±0.19	0	0	0	0.09±0.08
Mean fish size ±SE (*n*)	69.8±1.9 (32)	63.0±2.3 (19)	63.7±1.6 (51)	64.0±2.4 (17)	66.9±1.2 (24)

in soft-bottomed lakes are usually associated with vegetation. In Bassett, pumpkinseeds exhibited more sediment prey (i.e. gomphids and *Hexagenia*, Table 5) but still used more gastropods than the longear. Pumpkinseeds in Deep Lake were feeding on chironomids and gastropods (Table 5).

Diet similarity between large longear and pumpkinseed sunfish in midsummer computed as percent overlap (Schoener 1970) was only 19.5% in Hamilton and $\ll$10% in Shaw Lake. By contrast diet overlap was nearly 48% in sparsely vegetated Bassett Lake.

In general, longears used much larger food sizes than pumpkinseeds of similar size due principally to the greater use of large sediment prey. For instance, in Bassett Lake pumpkinseeds did not match 60- to 70-mm longears in average prey size until over 100 mm in length. Data from the spring of 1977 in Hamilton Lake indicated that the average prey size of pumpkinseeds (79.5 ± 1.2 mm) was 0.36 ± 0.04 mg while that of longears (69.8 ± 1.9 mm) was 1.58 ± 0.36 mg. Even pumpkinseeds above 100 mm in Hamilton Lake did not use prey of this size.

The handling time experiments indicated that longears of equivalent mouth gape could handle larger food sizes more efficiently than the pumpkinseed (Fig. 6). The difference in prey handling abilities at equivalent prey size/mouth gape ratios may be related to the pumpkinseeds' massive pharyngeal structure which is used to crush gastropods. This structure with its molariform teeth may be less efficient at "ratcheting" food back into the stomach than the smaller apparatus equipped with backward projecting teeth of the longear.

Sampling was generally inadequate to illustrate seasonal patterns in food use. There was a suggestion, however, that the average amount of food in the longear declined over the season (Fig. 7). Other studies have also shown that amount of invertebrate foods in the stomachs of temperate freshwater fish declines over

TABLE 5. Mean percent dry weight (±SE) of selected food items in the stomachs of pumpkinseed sunfish.

	Hamilton		Bassett	Deep Lake	Shaw
	May 8–12, 1977	Aug. 15, 1976	July 6, 1977	Aug. 7, 1977	Aug. 5, 1977
Libellulids	2.2±1.2	0.09±0.09	0	0	0
Gomphids	1.3±1.3	0	16.1±5.4	0	0
Aeschnids	0	0	0	0	0
Hexagenia	0.5±0.5	0	14.7±5.6	0	0
Coenagrionids	4.5±1.6	0	0	0	0
Caenis	10.5±3.2	0.1±0.1	2.3±1.8	2.9±0.9	0
Ephemerella	16.3±5.3	0	0	0	0
Hyallela	1.5±0.4	0	2.4±0.9	0.17±0.04	0
Chironomids	3.6±1.4	1.7±0.9	15.1±3.1	26.8±5.1	0.2±0.2
Helisoma	10.2±3.1	13.5±5.4	16.1±4.2	4.2±1.1	36.9±8.3
Amnicola	35.0±5.7	30.9±8.9	3.6±1.9	6.7±1.4	47.9±9.5
Physa	5.7±2.7	14.5±7.4	9.1±3.6	6.5±2.1	14.9±6.7
Mean fish size ±SE (*n*)	79.5±1.2 (28)	n.a. (13)	89.0±2.7 (31)	44.3±1.8 (9)	81.5±2.4 (21)

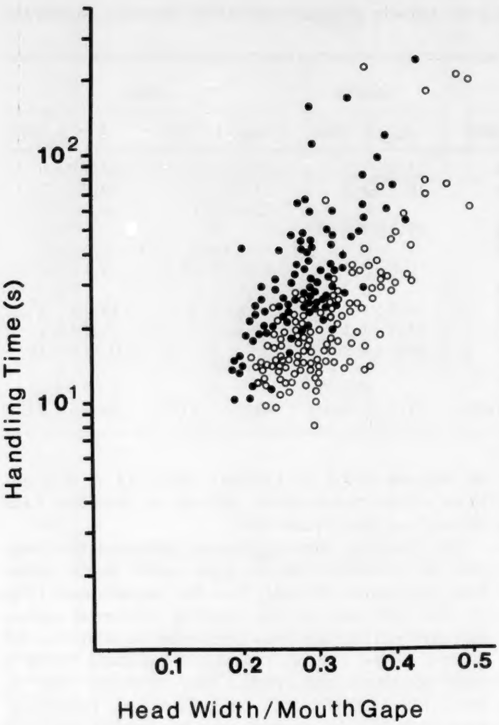


FIG. 6. Handling time per prey versus prey size/mouth gape for the longear (open symbols) and pumpkinseed (closed symbols) sunfish fed *Hexagenia* in the laboratory. Regressions for the two species differ at the 0.001 level.

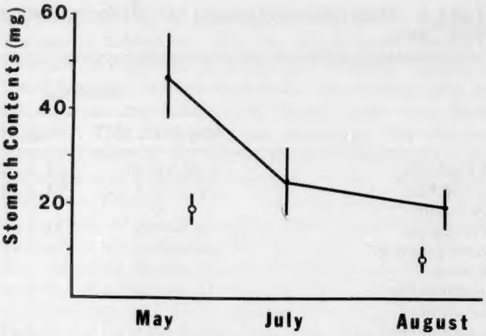


FIG. 7. Average amount of food (mg dry wt ± SE) in the stomachs of longear sunfish taken from Deep Lake (closed symbols) and Hamilton Lake (open symbols) in May, July, and August.

the growing season in small lakes (Seaburg and Moyle 1964; Ball 1948). There was further a suggestion that coenagrionid nymphs were more abundant in the diet of large longears in May than in July and August in Deep and Hamilton lakes where an early spring sample was available (Tables 3, 4). In Hamilton Lake pumpkinseeds contained more sediment prey in May, due principally to *Ephemerella* and *Caenis* and small numbers of libellulids. Gastropods, however, comprised the largest portion of the diet on all dates. Percent diet overlap between species increased in Hamilton during May to 38% versus the 19.5% exhibited by fish collected during August.

Discussion

Littoral zone habitat structure (i.e. plant cover and sediment type) greatly influenced the relative abundances of longears and pumpkinseeds. In fact, the longear sunfish is the only centrarchid in the small lakes in southern Michigan that exhibits a consistent presence/absence pattern correlated with a particular habitat type. It occurs almost exclusively in lakes with marl sediments and sparse stands of vegetation in the littoral zone. This pattern is unlikely related to lack of proper breeding sites. All *Lepomis* prefer firm substrates in shallow water with the longear breeding shallower than the others. Large areas of sandy or firm substrates did occur in shallow areas of the lakes in which the longear was absent. The pumpkinseed on the other hand is ubiquitous, occurring in all lake types and increasing in abundance as vegetation density and/or organic material of the sediments increases. The pumpkinseed is more generalized in its food and habitat use and exhibits considerable habitat switching in experimental studies (Werner and Hall 1976, 1979). Further, the food and habitat use data on the pumpkinseed in this study are only from a subset of the lakes/habitats that it inhabits (Keast 1965, 1978).

In general, food overlap between the pumpkinseed and longear appeared quite low (<10–50%), over a considerable range of vegetation densities and depths and less variable than spatial or macrohabitat overlap. The diet of the longear in particular was remarkably consistent across habitats within and between lakes. The differences in diet between the species further result in a considerable separation by food size. Average food size of the longear was often twofold larger than that of equivalent sized pumpkinseeds.

The above data indicate that the longear and pumpkinseed foraged different microhabitats; the larger longears used prey found in or on the sediments, notably large mayfly or odonate nymphs, whereas the pumpkinseed consumed much smaller prey (especially gastropods) which largely came from the vegetation. The pumpkinseed showed evidence of using substantial amounts of sediment prey only in Bassett Lake where vegetation was very sparse. Thus over a large gradient of vegetation densities, food overlap appears to be relatively insensitive to spatial overlap.

The prey used by the longear sheds some light on its association with open marl sediments as marl and organic sediments contain different resource sets. *Hexagenia limbata*, for instance, is absent from organic sediments (Hunt 1953) because of burrowing and respiration difficulties. In addition, Hunt (1953) found that *Hexagenia* actively avoided dense vegetation in the laboratory. Large burrowing dragonflies such as gomphids prefer grainy sediments, evidently for support (Corbet 1954), and both gomphid and libellulid dragonfly nymphs burrow or sprawl in the sediments which would be more difficult in organic flocs. Further, numerous authors cite the concentration of these large odonates in shallower water, especially prior to emergence (Kormondy 1959; Wright 1943). This may explain the shallow water distribution of large longears. A number of cores taken in the study lakes confirmed these general patterns. For instance, many fewer gomphids and no *Hexagenia* nymphs were collected in the organically rich sediments of Turner Lake or in organic regions of Hamilton Lake.

The longear evidently does not do well in organic or densely vegetated habitats. This may be due to the fact that behavioral as well as morphological adaptations are required to exploit efficiently the different prey that are found there. Different foraging tactics lead to capture of very different amounts of food within a habitat (Werner and Hall 1977; Ivlev 1961; Schutz and Northcote 1972) and the pumpkinseed and longear exhibit very different foraging behaviors. The longear is sedentary and remains very close to the sediments whereas the pumpkinseed is a much more active forager and ranges further from the sediments. A sedentary, sit-and-wait strategy may be required to key in on the slight movements of cryptic or burrowing sediment prey. These prey would be largely unavailable to the pumpkinseed searching vegetation above the sediments. Most snails used by the pumpkinseed in this study were closely associated with vegetation (Moyle and Bacon 1969; Townes 1938) which would preclude simultaneous searching for both gastropods and sediment prey. Werner and Hall (1977) make a similar argument for foraging tactic differences between the bluegill (*L. macrochirus*) and green sunfish (*L. cyanellus*). Such behavioral differences have often been related to species coexistence and relative success (Root 1967; Werner and Hall 1977).

Gastropods are very important in segregating these two species. At times upwards of 80–90% of the pumpkinseed diet is comprised of gastropods (Seaburg and Moyle 1964; Ball 1948; Keast 1965, 1978). Longears consumed very few gastropods in this study and will rarely accept snails when they are offered in aquaria. The pumpkinseed's large molariform pharyngeal plates enable it to crush hard-shelled prey thus providing considerable segregation from most of the other species in these systems (Werner et al. 1977). The increased use of odonates and mayflies in the spring when resources are abundant, and the preference for odonates and *Simocephalus* exhibited in pond studies (Werner and Hall 1976, 1979), however, suggest that gastropods are not the preferred food of the pumpkinseed but rather a competitive refuge.

The role of agonistic behavior in the interaction between these two species is unclear. Both intra- and inter-specific observations in the laboratory and during breeding in the field indicate that the longear is perhaps the most aggressive of the centrarchids in this community. Intraspecific aggression associated with feeding activity has been observed in field. However, many hours of underwater observation have indicated no overt aggressive behavior between these two species.

The analysis of resource use and competition between these two species is further complicated by the size-structure of the populations. Separation of adult fish is quite dramatic, but small longears and pumpkinseeds exhibit more extensive food and habitat overlap. This as well as previous studies have shown that the smaller size-classes of various species congregate in heavy vegetation presumably as a response to predation risk (Werner et al. 1977; Hall and Werner 1977). This could obviously lead to more intense competitive relations during this stage of the life history. Small pumpkinseeds may be faced with an additional constraint. Keast (1978) found that pumpkinseeds <77 mm in total length used molluscs only during the 1-mo period (July) in which small (young) gastropods occurred. The timing of small gastropod abundance is therefore important; if it occurs during periods when other food resources are abundant, its effect would be minimal. If it occurs when other resources are low, small pumpkinseeds would benefit.

Resource overlap therefore changes with fish size, and between size-classes changes seasonally. In some cases increased overlap is due to seasonal appearances of superabundant resources (Seaburg and Moyle 1964; Zaret and Rand 1971; Nilsson 1960) which lessen competition for resources. In other cases increased overlap may represent intense competition for resources and the potential for significant competitive bottlenecks. The increased overlap among small size-classes discussed above may well be a case in point, i.e. predation pressure may constrain habitat use to the point that the mechanisms responsible for niche segregation later in life cannot be expressed. Thus bottlenecks both during the life history or seasonally may have disproportionate effects on the regulation of population numbers or size distributions of the species populations. These considerations have received only cursory attention by fish ecologists and will be critical to understanding the patterns in community structure in fish.

The small differences in behavior and morphology between these two species lead to distinct quantitative differences in resource use when unconstrained by other factors (e.g. predation). Overlap in food was very low, for example, when adults were taken from the same habitat. This points to the subtle interactions between morphology and behavior of the predator and characteristics of the prey and its habitat. These interactions are ultimately responsible for the number of species that can coexist in lakes, and require much more detailed study.

Acknowledgments

We thank Donald Hall, Donald Beaver, Gary Mittelbach, James Gilliam, Leni Wilsmann, and Rich Merritt for valuable comments on an earlier draft. Robert Boling wrote the computer programs for analysis of the food habits. Barbara Laughlin provided field support and contributed in a host of other ways. Financial support was provided by NSF grants DEB 7620106, DEB 7704818, and DEB 7824271, and the Department of Zoology, Michigan State University. This is contribution No. 400 of the W. K. Kellogg Biological Station.

BALL, R. C. 1948. Relationship between available fish food, feeding habits of fish and total fish production in a Michigan lake. Univ. Mich. Tech. Bull. 206: 3–55.

CORBET, P. S. 1954. A biology of dragonflies. Quadrangle Press, Chicago, IL.

HALL, D. J., AND E. E. WERNER. 1977. Seasonal distribution and abundance of fishes in the littoral zone of a Michigan lake. Trans. Am. Fish. Soc. 106: 545–555.

HUBBS, C. L., AND G. P. COOPER. 1935. Age and growth of the long-eared and green sunfishes in Michigan. Pap. Mich. Acad. Sci. Arts Lett. 20: 669–696.

HUNT, B. P. 1953. The life history and economic importance of a burrowing mayfly, *Hexagenia limbata*, in southern Michigan lakes. Bull. Inst. Fish. Res. 4: 154 p.

IVLEV, V. S. 1961. The experimental ecology of the feeding of fishes. Yale Univ. Press, New Haven, CT. 302 p.

KEAST, A. 1965. Resource subdivision amongst cohabiting fish species in a bay, Lake Opinicon, Ontario, p. 106–132. Proc. 8th Conf. Gt. Lakes Res., Univ. Mich., Ann Arbor, MI.

1978. Feeding interrelations between age groups of pumpkinseed sunfish (*Lepomis gibbosus*) and comparisons with the bluegill sunfish (*L. macrochirus*). J. Fish. Res. Board Can. 35: 12–27.

KEENLEYSIDE, M. H. A. 1967. Behavior of male sunfish (genus *Lepomis*) towards females of three species. Evolution 21: 688–695.

KORMONDY, E. 1959. The systematics of *Tetragoneuria*, based on ecological, life history, and morphological evidence. Misc. Publ. Mus. Zool. Univ. Mich. 107: 1–79.

LAUGHLIN, D. R. 1979. Resource and habitat use patterns in two coexisting sunfish species (*Lepomis gibbosus* and *Lepomis megalotis peltastes*). Ph.D. thesis, Michigan State University, East Lansing, MI. 92 p.

MERRITT, R. W., AND K. W. CUMMINS. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa. 438 p.

MOYLE, P., AND J. BACON. 1969. Distribution and abundance of molluscs in a freshwater environment. J. Minn. Acad. Sci. 35: 82–85.

NILSSON, N. 1960. Seasonal fluctuations in the food segregation of trout, char and whitefish in 14 North-Swedish lakes. Rep. Inst. Freshwater Res. Drottningholm 41: 185–205.

PENNAK, R. 1954. Freshwater Invertebrates of the United States. Ronald, New York. 604 p.

ROOT, R. B. 1967. The niche exploitation pattern of the blue-gray gnatcatcher. Ecol. Monogr. 37: 317–350.

SCHOENER, T. W. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. Ecology 51: 408–418.

1974. Resource partitioning in ecological communities. Science 185: 27–39.

SCHUTZ, D. C., AND T. G. NORTHCOTE. 1972. An experimental study of feeding behavior and interaction of coastal cutthroat trout (*Salmo clarki clarki*) and dolly varden (*Salvelinus malma*). J. Fish. Res. Board Can. 29: 555–565.

SEABURG, K. G., AND J. B. MOYLE. 1964. Feeding habits, digestion rates, and growth of some Minnesota warm water fishes. Trans. Am. Fish. Soc. 93: 269–285.

SMITH, R. J. F. 1971. Control of prespawning behavior of sunfish (*Lepomis megalotis* and *Lepomis gibbosus*) II. Environmental factors. Anim. Behav. 18(3): 575–587.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. Freeman, San Francisco. 595 p.

TOWNES, H. K. 1938. Studies on the food organisms of fish. N.Y. State Cons. Dept. Biol. Surv. 12: 162–175.

TRAUTMAN, M. B. 1957. The fishes of Ohio. The Ohio State Univ. Press, Columbus, OH. 638 p.

WERNER, E. E., AND D. J. HALL. 1976. Niche shifts in sunfishes: experimental evidence and significance. Science 191: 404–406.

1977. Competition and habitat shift in two sunfishes (Centrarchidae). Ecology 58: 869–876.

1979. Foraging efficiency and habitat switching in competing sunfishes. Ecology 60: 256–264.

WERNER, E. E., D. J. HALL, D. R. LAUGHLIN, D. J. WAGNER. L. A. WILSMANN, AND F. C. FUNK. 1977. Habitat partitioning in a freshwater fish community. J. Fish. Res. Board Can. 34: 360–370.

WRIGHT, M. 1943. The effect of certain ecological factors on dragonfly nymphs. J. Tenn. Acad. Sci. 18: 172–196.

ZARET, T. M., AND A. S. RAND. 1971. Competition in tropical stream fishes: support for the competitive exclusion principle. Ecology 52: 336–342.

Models of Ammonia Excretion for Brook Trout (*Salvelinus fontinalis*) and Rainbow Trout (*Salmo gairdneri*)

LARRY J. PAULSON[1]

Division of Environmental Studies, University of California, Davis, CA 95616, USA

PAULSON, L. J. 1980. Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1421–1425.

Ammonia excretion by brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) was measured in relation to nitrogen consumption, body weight (15–154 g for rainbow trout and 50–360 g for brook trout), and temperature (11.2–21.0°C) under laboratory conditions. Four natural diets, collected from Castle Lake, California, and a commercial pellet diet were fed to the trout in gelatin capsules at feeding rates from 2.5 to 5% body weight · d^{-1}. Nitrogen consumption was the most important factor influencing ammonia excretion, followed by body weight and temperature. Testing the models with an independent data set revealed good agreement between measured and predicted rates of excretion. The models seem to estimate adequately ammonia excretion by trout in both natural and artificial aquatic systems.

Key words: models, ammonia excretion, nitrogen consumption, body weight, temperature, multiple regression, rainbow trout, brook trout

PAULSON, L. J. 1980. Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1421–1425.

L'excrétion ammoniacale chez l'omble de fontaine (*Salvelinus fontinalis*) et la truite arc-en-ciel (*Salmo gairdneri*) a été mesurée en fonction de la consommation d'azote, du poids corporel (15–154 g pour la truite arc-en-ciel et 50–360 g pour l'omble de fontaine) et de la température (11,2–21,0°C) dans des conditions de laboratoire. Les poissons ont été nourris à quatre régimes naturels recueillis dans le lac Castle, en Californie, et à un régime de boulettes commerciales, le tout dans des capsules de gélatine, à des taux d'alimentation de 2,5 à 5% du poids corporel·$jour^{-1}$. La consommation d'azote est le facteur qui exerce la plus grande influence sur l'excrétion ammoniacal, suivie du poids corporel et de la temperature. La vérification des modèles avec données indépendantes indique une bonne concordance entre les taux d'excrétion mesurés et prédits. Les modèles semblent estimer adéquatement l'excrétion ammoniacale dans des systèmes aquatiques, tant naturels qu'artificiels.

Received November 2, 1978
Accepted June 6, 1980

Reçu le 2 novembre 1978
Accepté le 6 juin 1980

AMMONIA is the principal end product of nitrogen metabolism by freshwater fish (Forster and Goldstein 1969). Although urea often comprises a significant part of total nitrogen excretion (Burrows 1964; Elliott 1976; Brett and Zala 1975), ammonia has greater ecological significance because it is an important plant nutrient, and in high concentrations can be toxic to aquatic animals.

When fish are confined at high density, as in hatchery or pond-rearing facilities, ammonia accumulation can impair growth and survival of fish stocks (Burrows 1964) and degrade water quality of effluents and receiving waters (Hinshaw 1973). However, since inorganic nitrogen is essential for phytoplankton, and ammonia is often assimilated preferentially over nitrate (Lui and Roels 1972), ammonia excretion by fish could be an important nitrogen source in natural systems. Quantitative models of ammonia excretion would be useful in establishing proper stocking density or water retention time in rearing facilities to avoid excessive ammonia accumulation, and in estimating ammonia excretion by fish in natural systems. The aim of this study, therefore, was to measure, and develop models of, ammonia excretion for both brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*).

Several factors influence ammonia excretion by fish. These include sex (Nightingale 1974), body weight (Savitz 1969; Infante 1974), rate of feeding (Savitz 1971; Brett and Zala 1975; Savitz et al. 1977), water temperature (Savitz 1969; Atherton and Aitken 1970; Maetz 1972), pH (Maetz 1973), and ambient ammonia concentrations (Fromm 1963; Fromm and Gillette 1968; Olson and Fromm 1971; Maetz 1973). Most measurements of rates of ammonia excretion (Infante 1974; Brett and Zala 1975) have been conducted in

---

[1] Present address: Lake Mead Limnological Research Center, University of Nevada, Las Vegas, NV 89154.

Printed in Canada (J5414)
Imprimé au Canada (J5414)

terms of each variable individually. However, Elliott (1976) found that excretory losses of brown trout (*Salmo trutta*) varied with temperature, body weight, and ration size. I measured ammonia excretion of brook and rainbow trout in relation to these three variables because they appear to be the most important factors influencing ammonia excretion by fish. This work was conducted as part of an investigation of ammonia regeneration by trout populations in Castle Lake, California (Paulson 1977).

Methods

ENVIRONMENT

Ammonia excretion measurements were conducted at the Castle Lake Limnological Research Laboratory, in a large tank separated into four equal compartments (61 × 61 × 48 cm) each of 178-L capacity. The system also included two 454-L holding tanks (61 × 61 × 122 cm); a 115-V submersible water pump; a 12-V recirculating pump; two cartridge filters (5 μm); and assorted pipe and plumbing fixtures, all 1.9-cm diameter. Inlet water was pumped from the lake (1-m depth) into the excretion tank through cartridge filters.

No efforts, except shading and insulation, were made to control the temperature in the tanks. Experiments were conducted throughout the summer and thus encompassed the range of natural conditions to which the trout would be exposed in the lake. Ambient ammonia, lake water, temperature, and pH ranged from 0 to 5 μg $N \cdot L^{-1}$, 11.2 to 21.0°C, and 7.0 to 7.2, respectively, during the period of study. Temperature in the tanks varied by 0.5–1.0°C, and ammonia increased to concentrations ranging from 50 to 200 μg $N \cdot L^{-1}$; there was no measurable change in pH during the 20- to 24-h excretion periods.

EXPERIMENTAL ANIMALS

Thirteen rainbow trout ranging in weight from 15 to 154 g and nine brook trout ranging from 50 to 360 g were captured by angling with barbless wet flies and lures. Sex was not determined because of sexual immaturity. The trout were confined in holding tanks and acclimated to laboratory conditions for several weeks before the experiments. During this period, they were trained to eat food contained in gelatin capsules. Training was facilitated by first feeding the trout meal worms.

DIET PREPARATION

Benthic invertebrates were collected from Castle Lake during the summer. In 1975, a composite benthic invertebrate diet was formulated with 63% Odonata, 32% Tricoptera, and 5% miscellaneous insects (dry weight). Benthic invertebrates collected in 1976 consisted of 69% Tricoptera, 20% Odonata, and 11% miscellaneous insects. A diet of terrestrial insects, collected by light traps and by hand, was prepared with a final composition of 30% Lepidoptera, 30% Coleoptera, 20% Ephemeroptera, 10% Diptera, and 10% miscellaneous insects. Sediment organic matter was collected from 3.5 m with a stratified bottom sampler, and the uppermost layer (2 cm) was used for diet preparation. Zooplankton were collected by repeated tows at 3 m with a Birge plankton net (80 μm). The approximate composition of this diet was 70% cladocerans (*Holopedium, Daphnia*) and 30% copepods (*Diatomus*). Commercial pellet food was also used as a diet (Sterling Feed Co.).

Each food source was dried at 60°C, ground in a porcelain mortar, and encapsulated in Nos. 2-, 3-, or 4-size gelatin capsules (Lilly). Ration size could thus be precisely controlled, and the possible chances of contamination by live food organisms were reduced. Gelatin capsules are readily hydrolyzed in the low pH medium of the stomach and do not impair diet digestion (Windell et al. 1969).

Trout were anesthetized, weighed, and then placed individually into three compartments of the excretion tank for several days before the experiments. One compartment served as a control. Prior to each experiment, the tanks were thoroughly flushed with filtered water to remove fecal and soluble wastes.

Each diet was fed to the trout at a rate of ~2.5 and 5.0% body weight$\cdot d^{-1}$, at 13:00 on the date of an experiment. Water was collected for ammonia determination at 09:00 the following day. The duration of the excretion periods ranged from 20 to 24 h. Several experiments were also conducted after the trout had been starved for several hours.

CHEMICAL ANALYSIS

Two litres of water was collected from each compartment of the excretion tank by siphoning. Duplicate ammonia determinations were made immediately on 50-mL subsamples using the phenol–hypochlorite method (Solorzano 1969).

Triplicate subsamples of each diet were analyzed for total nitrogen by combustion in a Hewlett Packard model 185B elemental analyzer according to the procedure described by Sharp (1974). Duplicate subsamples of the benthic invertebrate diet used in the 1975 experiments were analyzed for total nitrogen by Kjeldahl methods (APHA 1971).

AMMONIA EXCRETION MODELS

The rate of ammonia excretion was related to nitrogen consumption, body weight, and temperature for both species by the following equation:

$$(1) \quad E = ak^{Rb_1} + {}^{Tb_2} W^{b_3}$$

where:

a = constant,
k = 10,
R = nitrogen consumption ($g \cdot d^{-1}$),
T = temperature (°C),
W = fish body weight (g wet weight),
b_1, b_2, b_3 = regression coefficients,
E = ammonia excretion ($\mu g \cdot h^{-1}$).

Equation (1) was derived from a model of phosphorus excretion developed for zooplankton by Peters and Rigler (1973), and is similar to the equation used by Elliott (1976) to estimate excretory losses of brown trout (*Salmo trutta*). Feeding rate and nitrogen content of the diet were combined in the regression analysis as the nitrogen consumption variable. The regression coefficients were estimated on the log-transformed equation by stepwise, multiple regression (Dixon 1970).

TABLE 1. Results of stepwise multiple regression for rainbow trout (n = 127) and brook trout (n = 67) relating the log of ammonia excretion to nitrogen consumption, log of fish body weight, and temperature.

Variable	Regression coefficient	95% confidence limits of coefficient	Multiple correlation (R)	Coefficient of determination (R^2)	Increase in R^2
Rainbow trout					
Constant	1.123				
Nitrogen consumption	7.767	1.33	0.644	0.415	0.415
Body weight	0.616	0.174	0.804	0.646	0.231
Temperature	0.016	0.012	0.815	0.664	0.018
Brook trout					
Constant	0.977				
Nitrogen consumption	11.478	2.70	0.735	0.540	0.540
Body weight	0.467	0.278	0.768	0.589	0.049
Temperature	0.035	0.028	0.791	0.626	0.037

Results and Discussion

Nitrogen consumption was the most important variable influencing ammonia excretion by trout, explaining 41.5 and 54.0%, respectively, of the variation in the brook and rainbow trout regressions (Table 1). Since the rate of feeding and nitrogen content of the diets (Table 2) were treated collectively, it was not possible to partition the respective contributions to the R^2 value. However, regardless of diet or body weight, ammonia excretion by both species increased with increasing rates of nitrogen consumption (Fig. 1). Similar relationships have been reported for ammonia excretion by carp (*Cyprinus carpio*) (Infante 1974), and for total nitrogen excretion by bluegill (*Lepomis macrochirus*) (Savitz 1971) and largemouth bass (*Micropterus salmoides*) (Savitz et al. 1977). Moreover, Brett and Zala (1975) found that ammonia excretion by sockeye salmon (*Onchorynchus nerka*) peaked 4–5 h after feeding and then decreased to original levels within 24 h, parallel to the rate of gut evacuation. Similar patterns in total nitrogen excretion were reported for largemouth bass (Savitz et al. 1977) and carp (Infante 1974). In two determinations of daily excretion patterns, rainbow trout also showed pulses of ammonia excretion 7–8 h after feeding, but this decreased to basal levels within 46 h (Fig. 2). The pulses of ammonia and total nitrogen excretion that occur after feeding and the positive relationship between excretion and nitrogen consumption both indicate that nitrogenous wastes are derived primarily from food intake as was suggested by Brett and Zala (1975).

The contribution of body weight to the R^2 value dif-

TABLE 2. Total nitrogen content of various diets used in the excretion experiments. Results are expressed as percentage of dry weight ± 1 SD.

Diet	% nitrogen
Commercial pellets	8.7±0.50
Terrestrial insects	8.3±0.75
Benthic invertebrates[a]	7.6±0.31
Zooplankton	7.0±0.80
Sediment	1.8±0.09

[a] Diet used in 1975 and analyzed by Kjeldahl method was 7.1% nitrogen.

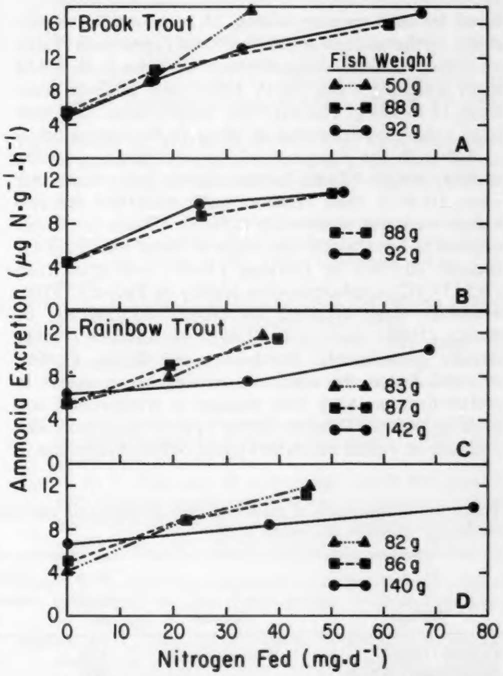


FIG. 1. Relationship between ammonia excretion and nitrogen consumption for various sizes of trout fed commercial pellet (A and C) and aquatic invertebrate diets (B and D) at ~2.5 and 5.0% body wt·d⁻¹. Experiments were conducted at a temperature range from 19.0 to 20.0°C for brook trout and 13.9 to 14.5°C for rainbow trout.

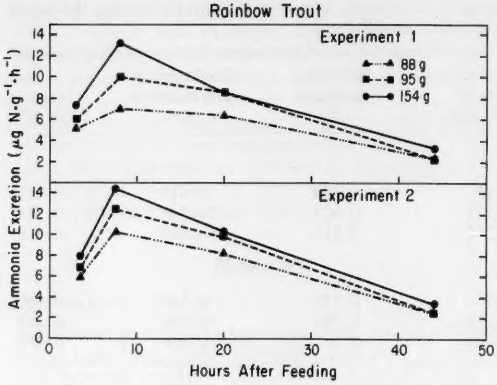


FIG. 2. Daily patterns of ammonia excretion for various sizes of rainbow trout fed a commercial pellet diet at ~2.5% body wt·d⁻¹. The same fish and feeding rates were used in both experiments. Water temperature ranged from 12.0 to 12.5°C.

fered for each species, adding 23.1 and 4.9%, respectively, in the rainbow and brook trout regressions (Table 1). Most excretion measurements were made on brook trout weighing from 50 to 100 g, and rainbow trout from 15 to 154 g. The narrower weight range for brook trout could have reduced its effect in the regression.

Although the exponent relating ammonia excretion to body weight (Table 1) was slightly lower for brook trout (0.467) than rainbow trout (0.616), the two values were not statistically different (Table 1). These exponents were within the range of those estimated for bluegill (0.530) by Gerking (1955) and grass carp (0.545) (*Ctenopharyngodon idella*) by Fisher (1970). However, those reported for bluegill (0.93–0.99) by Savitz (1969) and carp (0.902) by Infante (1974) deviate considerably. Paloheimo and Dickie (1966) concluded that the exponent relating body weight to metabolism can vary with changes in temperature and feeding regime. Thus, as Savitz (1969) suggested, differences in weight exponents could reflect differences in nutritional and thermal history of the experimental animals in the laboratory.

Temperature contributed little to the R^2 value adding only 1.8 and 3.7%, respectively, for rainbow and brook trout regressions (Table 1). The temperature range over which these experiments were conducted (11.2–21.0°C) appears adequate to detect a relationship if one were present. Savitz (1969) found that nitrogen excretion of bluegills at 7.2 and 15.6°C was similar and attributed this to a physiological adjustment of the fish to low temperature. This may partially explain the observations, but the thermal history of the experimental animals should also be considered in this instance. In this, and Savitz' (1969) study, the fish originated from natural stocks in temperate lakes that are subject to extreme seasonal temperature variation. Fish accustomed to these conditions could be expected to exhibit more rapid physiological adjustments to different thermal regimes in the laboratory than fish acclimated to constant conditions. This might explain why ammonia excretion by trout did not change appreciably with temperature in the range used in these experiments.

The transformed models of ammonia excretion for the two species are

$$\text{(2)}\quad \text{Rainbow trout} = 13.274\, k^{7.677(R)+0.016(T)}\, W^{0.616}$$

$$\text{(3)}\quad \text{Brook trout} = 9.484\, k^{11.478(R)+0.035(T)}\, W^{0.467}$$

The multiple R values, 0.82 for rainbow trout and 0.79 for brook trout, and the F-values were both significant ($P = 0.05$).

The models were tested against a set of independent data derived from other publications to determine their predictive value (Table 3). Although data were not available on nitrogen consumption, the comparison at starvation levels showed good agreement between actual measurements and predicted values from the regression models, even beyond the weight range for which the models were developed. Only at 20°C, for a 900-g rainbow trout, did the predicted values deviate substantially from the actual measurements (Table 3).

These models were adequate for estimating ammonia regeneration by the trout populations in Castle Lake,

TABLE 3. Comparison of measured rates of ammonia excretion from previous studies with those predicted from regression models for rainbow and brook trout.

Author	Species	Fish weight (g)	Nitrogen consumption (g)	Temp. (°C)	Measured excretion ($\mu g \cdot g^{-1} \cdot h^{-1}$)	Predicted excretion ($\mu g \cdot g^{-1} \cdot h^{-1}$)
Fromm (1963)[a]	Rainbow trout	129	0	13	3.1	3.3
Nightingale (1974)[a]	” ”	900	0	10	1.3	1.4
Nightingale (1974)[a]	” ”	900	0	15	2.3	1.7
Nightingale (1974)[a]	” ”	900	0	20	3.5	2.0
Morgulis (1918)[b]	Brook trout	96	0	16.5	3.6	3.2

[a] From summary in Brett and Zala (1975).
[b] Expressed as total nitrogen.

California (Paulson 1977), where ambient ammonia is low (0–5 $\mu g\ N\cdot L^{-1}$) and pH is almost neutral (7.0–7.2). However, since these factors influence ammonia excretion by fish, their application to other systems may be questionable. Fromm and Gillette(1968) reported that ammonia excretion by rainbow trout decreased with increasing ammonia from 0 to 8 $mg\cdot L^{-1}$ but did not change significantly from 0 to 3 $mg\cdot L^{-1}$. Maetz (1973) has shown that an abrupt decrease in pH can significantly increase ammonia excretion by goldfish (*Carassius auratus*) by altering the internal/external ammonia gradient. The goldfish, however, rapidly adjusted to the shift in pH since their excretion rates decreased to original levels within a few hours. Thus, moderately high concentrations of ambient ammonia and abrupt changes in pH produce only minimal or short-term changes in ammonia excretion. Therefore, the regression models should provide reasonable estimates of ammonia excretion by trout in other systems, even though ambient ammonia and pH were not included as variables.

Acknowledgments

I thank C. R. Goldman and P. E. Kellar for reviewing the manuscript and R. P. Axler for assisting with construction of the excretion tanks. I am also grateful to B. L. Kimmel and B. Allardice for performing the diet nitrogen analysis, and to S. Paulson and D. Edelman for assistance in preparing the manuscript. This work was supported by National Science Foundation grants BM74-02246 A01 and DEB76-19524 to C. R. Goldman.

AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. AMERICAN PUBLIC HEALTH ASSOCIATION. (APHA) 1971. Standard methods for examination of water and wastewater. 13th ed. 847 p.

ATHERTON, W. D., AND A. AITKEN. 1970. Growth, nitrogen metabolism, and fat metabolism in *Salmo gairdnerii* Richardson. Comp. Biochem. Physiol. 36: 719–747.

BRETT, J. R., AND C. A. ZALA. 1975. Daily patterns of nitrogen excretion and oxygen consumption of sockeye salmon (*Onchorynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 32: 2479–2486.

BURROWS, R. E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. U.S. Fish. Wildl. Ser., Bur. Sport Fish. Wildl. Res. Rep. 66: 12 p.

DIXON, W. J. [ed.] 1970. Biomedical computer programs. Univ. Calif. Press, CA. 600 p.

ELLIOTT, J. 1976. Energy losses in the waste products of brown trout (*Salmo trutta* L). J. Anim. Ecol. 45: 561–580.

FISHER, Z. 1970. The elements of energy balance in grass carp, (*Ctenopharyngodon idella* Vah.) Part 1. Pol. Arch. Hydrobiol. 17: 421–434.

FORSTER, P. P., AND L. GOLDSTEIN. 1969. Formation of excretory products, p. 313–345. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology, Vol. 1. Excretion, ion regulation, and metabolism. Academic Press, NY. 455 p.

FROMM, P. O. 1963. Studies on renal and extra-renal excretion in a freshwater teleost, *Salmo gairdneri*. Comp. Biochem. Physiol. 10: 121–128.

FROMM, P. O., AND J. R. GILLETTE. 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*) Comp. Biochem. Physiol. 26: 887–896.

GERKING, S. D. 1955. Endogenous nitrogen excretion of bluegill sunfish. Physiol. Zool. 28: 283–289.

HINSHAW, R. N. 1973. Pollution as a result of fish cultural activities. Env. Prot. Agency, Ecol. Res. Ser. Rep. EPA-R3-73-009: 209 p.

INFANTE, V. O. 1974. Untersuchungen uber die stickstoffexkretion junger karpfen (*Cyprinus carpio*) im hunger und bei futtersung. Arch. Hydrobiol. Suppl. 47: 239–281. (English summary)

LUI, N. S., AND O. A. ROELS. 1972. Nitrogen metabolism of aquatic organisms. II. The assimilation of nitrate, nitrite, and ammonia by *Biddulphia aurita*. J. Phycol. 8: 259–264.

MAETZ, J. 1972. Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus*. Effects of ammonia-loading and temperature changes. J. Exp. Biol. 56: 601–620.

1973. Na^+/NH_4^+, Na^+/H^+ exchanges and ammonia movement across the gills of *Carassius auratus*. J. Exp. Biol. 58: 255–275.

MORGULIS, S. 1918. Studies on the nutrition of fish. J. Biol. Chem. 36: 391–413.

NIGHTINGALE, J. W. 1974. Bioenergetic responses of nitrogen metabolism and respiration to various temperatures and feeding intervals in Donaldson strain rainbow trout. Ph.D. thesis, Univ. Washington, St. Louis, MO. 110 p.

OLSON, K. R., AND P. O. FROMM. 1971. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp. Biochem. Physiol. 40: 999–1007.

PALOHEIMO, J. E., AND L. M. DICKIE. 1966. Food and growth of fishes. II. Effects of food and temperature on the relation between metabolism and body weight. J. Fish. Res. Board Can. 23: 869–908.

PAULSON, L. J. 1977. The significance of ammonia regeneration by trout on phytoplankton productivity in Castle Lake, California. Ph.D. thesis, Univ. California, Davis, CA. 183 p.

PETERS, R. H., AND F. H. RIGLER. 1973. Phosphorus release by *Daphnia*. Limnol. Oceanogr. 18: 821–839.

SAVITZ, J. 1969. Effects of temperature and body weight on endogenous nitrogen excretion in the bluegill sunfish (*Lepomis macrochirus*). J. Fish. Res. Board Can. 26: 1813–1821.

1971. Nitrogen excretion and protein consumption of the bluegill sunfish (*Lepomis macrochirus*). J. Fish. Res. Board Can. 28: 449–451.

SAVITZ, J., E. ALBANESE, M. J. EVINGER, AND P. KOLASINSKI. 1977. Effect of ration level on nitrogen excretion, nitrogen retention and efficiency of nitrogen utilization in largemouth bass (*Micropterus salmoides*). J. Fish. Biol. 11: 185–192.

SHARP, J. H. 1974. Improved analysis of "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr. 19: 984–989.

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14: 799–801.

WINDELL, J. T., D. O. NORRIS, J. F. KITCHELL, AND J. S. NORRIS. 1969. Digestive response of rainbow trout, *Salmo gairdneri* to pellet diets. J. Fish. Res. Board Can. 26: 1801–1812.

Some Sources of Variability in Determination of Digestive Enzyme Activity in Zooplankton

P. MAYZAUD[1,2]

Fisheries and Marine Service, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

MAYZAUD, P. 1979. Some sources of variability in determination of digestive enzyme activity in zooplankton. Can. J. Fish. Aquat. Sci. 37: 1426–1432.

Enzyme activity measurements, sometimes used to ascertain the distribution and quantification of physiological activity of marine zooplankton, are sensitive to a number of sources of variability of both methodological and environmental origins. To facilitate multiple enzyme determinations on a single sample, we have developed a method of acetone extraction from neritic zooplankton followed by 50% glycerol homogenization of the resulting powder. An increase in activity was found compared with that of glycerol homogenates of live zooplankton. Because saturation of 50% glycerol was found when extracting increasing amounts of acetone powder, care was taken to prepare homogenates with an identical amount of powder. The problem of protein solubility in the homogenization medium is discussed. The method is repeatable and yields results which are little influenced by short-term storage of animals before extraction but are strongly influenced by species composition of the plankton sample and to a lesser extent by spatial variability of the plankton populations.

Key words: variability, determination, digestive enzyme activity, zooplankton

MAYZAUD, P. 1979. Some sources of variability in determination of digestive enzyme activity in zooplankton. Can. J. Fish. Aquat. Sci. 37: 1426–1432.

Les mesures d'activité enzymatique, parfois utilisées pour définir la répartition et quantifier l'activité physiologique du phytoplancton marin, sont sensibles à plusieurs sources de variabilité, tant dans la méthode que dans l'environnement. Afin de faciliter plusieurs déterminations d'enzymes sur un même échantillon, nous avons mis au point une méthode d'extraction à l'acétone à même du zooplancton néritique, suivie d'une homogénéisation au glycérol à 50% de la poudre résultante. Une activité accrue a été observée par comparaison avec celle d'homogénats au glycérol de zooplancton vivant. Parce qu'on avait observé une saturation du glycérol à 50% lors de l'extraction de quantités croissantes de poudre d'acétone, on s'est assuré que les homogénats étaient préparés avec une quantité identique de poudre. Nous analysons le problème de la solubilité des protéines dans le milieu d'homogénéisation. La méthode peut se répéter et donne des résultats qu'un entreposage à court terme des animaux avant extraction affecte très peu. Par contre, la composition par espèce de l'échantillon de plancton les affecte considérablement; il en est ainsi, bien qu'à un degré moindre, de la variabilité spatiale des populations planctoniques.

Received June 5, 1979
Accepted June 5, 1980

Reçu le 5 juin 1979
Accepté le 5 juin 1980

THERE is a critical need for simple analytical techniques to ascertain the distribution and quantify the physiological activity of marine zooplankton in their environment. Recent work by King and Packard (1975), Boucher and Samain (1974), Boucher et al. (1976), Mayzaud and Conover (1976), and Mayzaud and Poulet (1978) have demonstrated the feasibility of using enzyme methods to quantify physiological activity such as respiration or nutrition. However, to make these methods generally applicable there are a number of methodological and environmental problems which must be considered. Among the important ones are the preservation of the enzymatic activity during the preparation and storage of the plankton samples and, when total populations are studied, the heterogeneity of the species composition of the samples and the environment.

Handling and storage of cell and tissue enzymes from animals and microorganisms of terrestrial origin have been reviewed by Hess and Brand (1974) but little has been published as yet for marine zooplankton. Usually enzyme samples are prepared either as wet homogenates by grinding in buffer or distilled water, by sonic or thermal homogenization, or as dry homogenates after acetone dehydration or freeze-drying. Whenever the enzyme analysis cannot be run immediately, deep freezing (−60°C) of the tissue samples or homogenates and storage at the same temperature

---

[1] Present address: Biochimie Marine, Station Zoologique, 06230 Villefranche-sur-Mer, France.

[2] B I O contribution.

Printed in Canada (J5659)
Imprimé au Canada (J5659)

TABLE 1. Assay conditions for poly- and di-saccharidases.

Substrate concn. % (V/V)	Substrate vol. used (mL)	Substrate pH	Enzyme–glycerol extract (μL)	Incubation time (min)	Incubation temp. (°C)
Maltose 1%	0.5	5.0	75	15	35
Cellobiose 1%	0.5	5.5	75	15	35
Lactose 15%	0.5	5.0	150	60	35
Trehalose 1%	0.5	6.0	150	60	35
Laminarin 1%	0.5	5.0	75	15	35
Carboxymethyl cellulose 1%	0.5	4.5	100	60	35

is usually a method of choice for both phytoplankton and zooplankton (Ahmed et al. 1976). However, when several enzymatic activities must be determined on the same sample, dry homogenates provide a safer storage of the material beween assays than the freezing procedure. Before adopting such procedures, thorough examination of their applicability to the zooplankton and their sources of variability are necessary.

Although a large variance is known to exist in plankton sampling generally (Platt and Conover 1971), little attention has been given to the different sources of variability introduced by both species diversity within a given sample and spatial heterogeneity of populations within a given environment. These different factors are bound to introduce potential errors if not taken into account in the definition of the sampling and handling procedures.

Methods

Zooplankton was usually captured with a No. 6 net (240-μm mesh) and then passed through 800-μm mesh bolting-cloth to remove large carnivores (chaetognaths, jellyfish, etc.). It was then rinsed a second time on No. 6 mesh to remove the phytoplankters. To minimize mortality, care was taken to ensure that the plankton was never permitted to become dry during the sieving and rinsing. Finally excess water was drained by the use of filter paper or facial tissue and the animals were transferred rapidly into high purity or redistilled acetone (−40°C) before being blended at 0°C with a Sorvall Omnimixer in proportions of 100 mL of acetone to a maximum of 5 g of plankton. The mixture was then transferred into an Erlenmeyer flask and the grinding vessel was rinsed with an additional 100 mL of cold acetone. The flask containing the combined acetone extracts was left for 90 min in crushed ice to ensure complete dehydration. After filtration, the residue was again treated with the same amount of acetone at room temperature for 10 min, filtered, transferred to small glass vials, and dried under vacuum at −20°C in a desiccator; the resulting material is henceforth called "acetone powder." Faster drying can be obtained by additional rinsing with anhydrous ether (Prosser and Van Weel 1958), but this can denature certain enzymes and should be tested before adoption.

Acetone powder from zooplankton was extracted with distilled water or 50% glycerol at 5°C overnight in proportions of 1 mL of glycerol to 5 mg of powder. As found by Prosser and Van Weel (1958), extraction with distilled water alone was not adequate. Enzyme activities were assayed on aliquots of glycerol supernatant after centrifugation at 800–1000 g at 0°C.

Amylase activity was measured according to the ultramicro procedure described by Street and Close (1956). The reactive medium was adjusted to pH 6.0 with 0.1 mol/L phosphate buffer and incubated at 30°C for 10 min with 40 μL of glycerol enzyme solution. The reaction was stopped by addition of 0.6 mL of 0.01 mol/L iodine solution and quickly diluted to 10 mL with distilled water. As indicated by Mayzaud (unpublished data) optimal activation was obtained by addition of 0.1 mol/L NaCl to the substrate. The activity was expressed as mg of amylose degraded per unit of time.

Disaccharidase, cellulase, and laminarinase were measured according to a procedure derived from that described by Dahlqvist (1964) taking advantage of the inhibition of disaccharidases by Tris buffer used in the glucose oxydase peroxidase reagent preparation. Laminarinase measurement was adapted from Vacquier (1971). Activities were expressed as μg of glucose produced per unit of time after measurement by the glucose–oxydase–peroxidase reagent dissolved in Tris buffer (0.1 mol/L, pH 7.0). Conditions of the various assays are given in Table 1.

Alkaline and acidic protease activities were measured by a modification of the technique described by Anson (1939) and were expressed as μg of tyrosine produced per unit time. I measured acidic protease using 0.5% urea-denatured hemoglobin as a substrate and alkaline protease using 0.5% bovine serum albumin. In both cases 150 μL of glycerol extract was added to 1 mL of buffered substrate (pH 4.5 for acidic protease and pH 9.5 for alkaline protease) and incubated at 40°C for 30 min. The reaction was stopped with 2 mL of 5% cold TCA (trichloroacetic acid) and the tubes were left at room temperature for 30 min. The precipitate was then centrifuged at 10 000 *g* for 20 min or filtered through high retention filter paper (Whatman 42). To 1 mL of supernatant, 1 mL of copper reagent was added (10 parts of 10% sodium carbonate in 0.5 mol/L NaOH and 1 part of 0.5% $CuSO_4$ in 1% sodium potassium tartrate). After 10 min at room temperature, 3 mL of diluted (1 to 11) Folin reagent was added and the coloration developed for 10 min at 50°C in a water bath. The tubes were cooled at room temperature and read at 750 nm using tyrosine as a standard (0.2 mL of 10^{-3} mol/L tyrosine in 0.8 mL of 0.2 mol/L HCl).

For all enzyme activity measurements, proper blanks and controls were run simultaneously, including a mixture of substrate and inactivated enzyme (boiled 10 min).

To compare with the acetone extraction procedure, homogenates of live copepods were also prepared in either 50% glycerol or appropriate buffer. Samples were then

TABLE 2. Comparison of enzyme specific activities between glycerol homogenization and acetone powder extraction from copepods ± SD.

Enzyme	Activity; glycerol homogenate	Activity; powder extracted in glycerol	Units
Amylase	13.26±0.50	11.28±0.34	mg amylose · mg Prot^{-1} · h^{-1}
Laminarinase	954.67±25.10	1054.83±25.64	μg glucose · mg Prot^{-1} · h^{-1}
Cellulase	102.15±0.27	42.27±0.36	μg glucose · mg Prot^{-1} · h^{-1}
Maltase	445.79±1.06	464.54±1.97	μg glucose · mg Prot^{-1} · h^{-1}
Cellobiase	402.83±5.53	524.86±5.74	μg glucose · mg Prot^{-1} · h^{-1}
Trehalase	83.61±2.28	77.09±2.72	μg glucose · mg Prot^{-1} · h^{-1}
Lactase	11.56±0.17	123.06±1.98	μg glucose · mg Prot^{-1} · h^{-1}
Acidic protease	2.61±0.33	2.92±0.28	μg Tyr · mg Prot^{-1} · min^{-1}
Alkaline protease	2.22±0.06	2.67±0.20	μg Tyr · mg Prot^{-1} · min^{-1}

centrifuged at 4000 *g* for glycerol homogenates or filtered through cheesecloth for buffer homogenates and the remaining supernatant or filtrate was used for enzymatic assays as described previously.

The protein content of glycerol and buffer extracts was measured using the method of Lowry et al. (1951), appropriately diluted (usually 5%) to give optimum color development and minimum interference by glycerol.

Statistical significance of the means was ascertained by a *t*-test.

Results and Discussion

COMPARISON BETWEEN ENZYME ACTIVITIES OF GLYCEROL HOMOGENATES AND ACETONE POWDER EXTRACT

To ensure that the acetone extraction procedure did not strongly inactivate the digestive enzymes, parallel determinations were run on paired aliquot fractions of the same copepod sample. Half were acetone-treated before being homogenized in 50% glycerol and the others directly homogenized in 50% glycerol. In each case three different samples were prepared and analyzed in triplicate. Except for cellulase, fair agreement was obtained between the methods (Table 2). Amylase showed a slight decrease in activity (15%) whereas the other enzymes presented a significant increase ($P > 0.05$) for the glycerol–acetone extract. This procedure appears to concentrate slightly the enzyme proteins versus the structural ones. In the case of cellulase, 60% of the activity disappeared during the extraction process and a more realistic measurement can probably be carried out using distilled water or buffer extracts. For both amylase and cellulase the problem remains to determine whether there is inactivation by the acetone extraction or lack of complete extraction of the protein molecules in the glycerol medium. In the case of amylase the difference is small and consistent (SD = 0.34) and does not prevent the use of the method.

EXTRACTABILITY OF ENZYMATIC PROTEIN FROM ACETONE POWDER

The acetone treatment results in a powder containing zooplankton proteins of which only a small number are digestive enzymes. Before deciding on a standard amount of powder to extract with glycerol one should make sure (1) that the powder contains a constant proportion of the different enzymes and (2) that increasing amount of powder yields linearly increasing enzyme activity. Linearity is obtained for most enzymes between 2 and 7 mg of homogenized powder (Fig. 1 and 2). Except cellobiase, all enzymes showed a reduced activity between 0 and 1 or 2 mg of powder which is possibly related to the small proportion of active protein compared to the total amount in the powder sample.

The degree to which a constant proportion of different enzymes is extracted is somewhat more difficult to ascertain. A covariance analysis of the slopes of the regressions between the amount of powder extracted

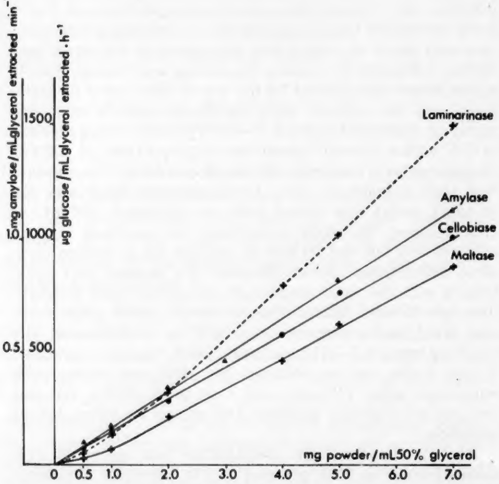


FIG. 1. Variations in amylase, laminarinase, cellobiase, and maltase activities with increasing amount of acetone powder homogenized in 50% glycerol.

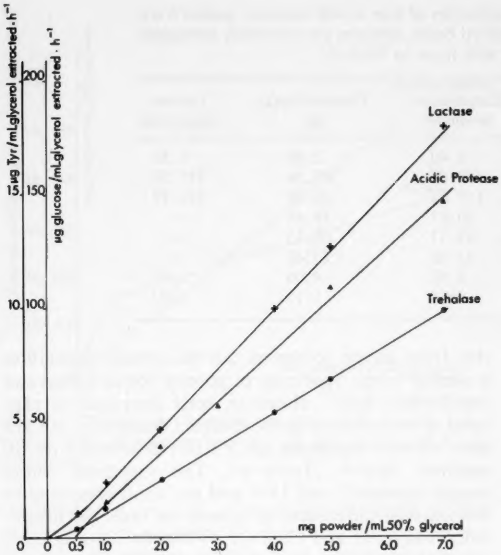


FIG. 2. Variations in lactase, trehalase, and acidic protease with increasing amounts of acetone powder homogenized in 50% glycerol.

and the enzyme activities should be used (Reeve test), but the lack of common units prevents its use for the set of enzyme curves presented here. Even without statistical demonstration it is clear from Fig. 1 and 2 that laminarinase has the highest slope and lactase and trehalase the lowest one. Cellobiase and maltase have a somewhat similar slope but a covariance analysis showed that they are not truly parallel ($F_{1,4} = 70.3$). No conclusion can be drawn for amylase and protease, but it seems evident that the more powder extracted, the more acivity will be recorded for enzymes such as laminarinase and to a lesser extent maltase and cellobiase as compared to trehalase and lactase. When comparisons must be made between the different populations, no consistent results can be expected if different quantities of powder are extracted. To overcome this problem, we used routinely 5 mg of powder per millilitre of 50% glycerol, but any value between 2 and 7 mg of powder could be used.

To ascertain that 50% glycerol always solubilized the same amount of enzyme protein from 5 mg of powder, five different extractions of samples of the same crude powder were made. The results confirmed that little variability was introduced by powder heterogeneity at the 5-mg level (Table 3). One homogenate per sample was therefore considered sufficient to represent the average enzymatic activity of the extracted powder.

TABLE 3. Repeatability of enzyme measurement for five different 50% glycerol homogenizations on the same acetone powder extract (for activity see Table 2).

Enzyme	Activity	SD	Coefficient of variation (%)
Amylase	49.98	1.08	2.1
Laminarinase	1026.56	40.69	4.2
Maltase	668.59	13.68	2.0
Cellobiase	756.13	12.48	1.7
Trehalase	68.08	2.09	3.1
Lactase	125.06	4.15	3.3
Acidic protease	1.21	0.05	4.1

INFLUENCE OF ZOOPLANKTON PHYSIOLOGICAL STATE

One of the difficulties in extrapolating laboratory measurements to the natural environment is estimating the error introduced by sampling and handling procedures. To examine this effect, a tow containing only copepods was first diluted in an insulated plastic container and then divided into two lots. Lot I was extracted with acetone immediately on board the ship and lot II was left in the cooler in a cold room at in situ temperature (5°C) for 6 h without any special treatment, after which the live animals were also acetone-extracted. Except for laminarinase, the second bath (II) had similar or higher enzyme activities when compared with the first one (I) (Table 4). If serious enzyme degradation had taken place during the 6 h of storage, lower activities for more than one enzyme would have been expected.

In fact the slight increase of activity for four enzymes out of eight in batch II is quite puzzling. It may correspond either to a change in physiological activity during the 6-h storage or to a slight modification in the species composition of the population caused by differential mortality.

INFLUENCE OF ZOOPLANKTON SPECIES COMPOSITION

As indicated by Conover and Mayzaud (1976) five major copepod species dominated the zooplankton

TABLE 4. Influence of time of storage on enzyme specific activities of freshly caught zooplankton (I); and after 6 h of storage in a cooler at 5°C (II). $n = 3$.

Enzyme	Activity ± SD (I)	Activity ± SD (II)
Amylase	11.28±0.34	11.10±0.08
Laminarinase	1054.83±15.64	759.93±9.31
Maltase	421.69±1.97	507.86±2.16
Cellobiase	524.86±5.74	571.25±5.20
Trehalase	51.09±1.68	83.09±2.72
Lactase	123.06±2.08	128.97±2.11
Acidic protease	1.73±0.09	1.67±0.10
Alkaline protease	2.76±0.10	2.34±0.10

TABLE 5. Carbohydrase and protease specific activities of four neritic copepod species from the same tow caught on July 12, 1978 in the Bedford Basin. Samples are essentially composed of copepodid V and adults. Units are identical with those in Table 2.

Enzyme	*Acartia hudsonica*	*Eurytemora herdmani*	*Pseudocalanus* sp.	*Temora longicornis*
Amylase	12.72	5.40	2.46	3.30
Laminarinase	965.78	430.67	506.59	927.58
Cellulase	194.79	157.64	29.19	349.79
Maltase	187.19	80.85	59.44	—
Cellobiase	107.93	83.32	68.13	—
Trehalase	34.46	53.30	23.43	—
Acidic protease	3.17	3.68	4.06	5.65
Alkaline protease	2.34	2.05	1.13	2.92

captured with a No. 6 net during the summer months. Four of these species displayed different levels of activity for the carbohydratases and proteases considered (Table 5), which may be related to the kind of particulate matter on which they feed.

For example, in July, only *Acartia* derived a large fraction of its energy from α-(1–4) and β-(1–3) polysaccharides. *Temora* had a much lower amylase activity than *Acartia* but had active laminarinase and cellulase. It also had the most active proteases. *Eurytemora* and *Pseudocalanus* showed intermediate patterns with relatively high acidic protease and laminarinase, but before generalizing from such results, it should be pointed out that the respective activities also may change significantly throughout the seasons (Mayzaud unpublished data). In any case, when total population activities are studied, care should be taken to extract the whole tow or to ensure its homogeneity before subsampling in order to reduce the variability introduced by species differences.

SPATIAL HETEROGENEITY OF TOTAL POPULATION ENZYMES

Spatial heterogeneity of plankton populations has been well established (see review by Platt 1975) and might be expected to introduce significant variability in enzyme measurements of total zooplankton populations. Small variations in species composition are noticeable from station to station but the overall population is similar in the spectrum of species found (Mayzaud unpublished data). However, total zooplankton captured at two stations in the Bedford Basin only 0.8 km apart showed significant ($P > 0.05$) differences for all enzymes studied (Table 6). The variations found ranged between 5 and 25% and are small compared to the seasonal differences of almost an order of magnitude (Mayzaud and Conover 1976). In more oceanic environments where seasonality may be less important, spatial heterogeneity may be a major source of variations when sampling is carried out over relatively short periods of time.

PROTEIN CONTENT AND ENZYME ACTIVITY

To obtain comparable results, most authors reporting digestive enzyme activity of crustaceans (see review by Van Weel 1970) or zooplankton (Boucher and Samain 1974, 1975; Boucher et al. 1976; Mayzaud and Conover 1976; Mayzaud and Poulet 1978) have usually divided their unit of enzyme activity by the protein content of their crude homogenate and called it specific activity. As indicated by Mayzaud and Poulet (1978) this measurement is not necessarily equivalent to true specific activity. Using total protein instead of enzyme protein to compute a specific activity is to assume that enzyme protein and total protein are

TABLE 6. Comparison of enzyme specific activities of two copepod populations taken at two different stations (see text). Units are identical with those in Table 2. n = number of determinations. For station location see Platt and Conover (1971). *$P > 0.05$; **$P > 0.01$.

Enzyme	Activity, Station 2	Surface	Activity, Station 1	Surface
Amylase	28.9±0.9	(4)	32.3±0.7	(4)**
Laminarinase	1730.2±13.5	(3)	1649.4±19.2	(3)**
Maltase	690.01±1.80	(3)	853.5±2.08	(3)**
Cellobiase	763.08±4.34	(3)	1029.28±4.94	(3)**
Trehalase	33.87±3.02	(3)	40.26±1.13	(3)*
Lactase	98.91±0.88	(3)	147.89±0.62	(2)**
Acidic protease	8.80±0.18	(3)	10.73±0.71	(3)*
Alkaline protease	4.19±0.28	(3)	3.95±0.08	(3)**

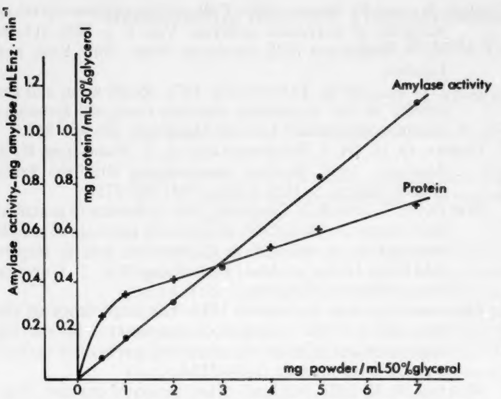


FIG. 3. Changes in amylase activity and protein solubilized with increasing quantity of acetone powder homogenized.

extracted in a constant ratio regardless of the weight of powder homogenized. To check this assumption the variations in amylase activity were compared with increasing quantity of crude powder and the amount of total protein extracted. Though amylase activity was linearly related to the quantity of crude powder, the extraction of total protein showed a tendency toward saturation above a concentration of 1 mg of powder per millilitre (Fig. 3). Under such conditions it is obvious that dividing the unit of enzyme or activity by the protein content would lead to an increase in variability rather than a decrease. For this reason and others already seen, all measurements must be carried out with homogenates containing the same concentration of crude powder. Corrections for variation in the protein content of the glycerol extract were possible, because over the narrow range observed (10 to 20%) the enzyme activity was linearly related to the protein measured. Under such conditions use of specific activities was appropriate and variability was reduced.

Why protein was not extracted in a linear fashion is very probably a function of solubility in glycerol which may be either a property induced by the acetone extraction or by the medium used to extract the acetone powder. Comparison of the extraction process on live copepods in either 50% glycerol or distilled water (Fig. 4) suggests that the extraction medium is probably at fault. The glycerol curve, though linear, does not intercept the origin. Whether this means that glycerol interference with copper–Folin coloration is stronger at low animal densities remains to be shown. Since standards were also prepared in glycerol, this assumption would imply that other interfering compounds were selectively extracted by 50% glycerol. Whatever the reason, working with homogenates containing a similar number of zooplankton per unit of volume will be necessary to get comparable results. Additional data obtained by S. Dallot (SZV-Villefranche-sur-Mer, personal communication) on chaetognaths homogenized in distilled water showed (1) that for large animals the ratio of protein to weight varies and thus might introduce a substantial error into enzymatic measurements, even for a constant number of individuals; and (2) that for homogenates ranging between 5 and 20 chaetognaths, 90% of the variance shown by the digestive enzyme activity (protease) was explained by the variations in number of animals in the homogenate. This also suggests that even when using distilled water for homogenization care must be taken that enzyme protein and total protein follow the same trend of variation.

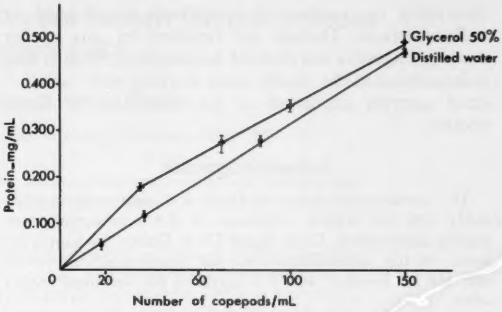


FIG. 4. Protein solubilization in 50% glycerol and distilled water from various numbers of live copepods. (Three different homogenizations were used.)

Conclusion

Digestive enzymes are not usually very difficult to extract in aqueous solution if certain percautions are taken (Morton 1955). As indicated earlier, three different methodologies can be used: (1) direct extraction of live animals in either distilled water or buffer; (2) deep-freezing followed by freeze-drying; and (3) solvent extraction. Each of these methods has advantages and drawbacks and the choice should be based upon the facilities, the type of experiment, and the kinds of enzymes considered. The method used here was found to be suitable for total population studies. Various enzymes can be studied on the same sample without inactivation and the dry powder is stable at −20°C for many months (up to 1 yr, unpublished data). Nevertheless, when small samples of sorted species and stages are required, deep-freezing is more convenient, though less practical for multienzyme determinations.

Of the different sources of variability studied here, three appeared to be very important and may influence conclusions based on ecological distribution of populations or enzyme activity. Variations in species composition and spatial heterogeneity of plankton distribution were expected factors which cannot usually be controlled in field studies. Very different in nature is the variability introduced by changes in protein concentration or number of individuals in glycerol or buffer extracts. Though not reported by any earlier work on digestive enzymes of zooplankton, serious bias is introduced in the results when working with variable-sized samples expressed as per milligram of tissue protein.

Acknowledgments

The continuous support of Dr R. J. Conover during this study and his helpful criticisms of the manuscript were greatly appreciated. I also thank Dr S. Dallot for supplying some of his unpublished data on chaetognath proteases and Mr M. Huntley and J. Chiaverini for technical assistance.

AHMED, S. I., R. A. KENNER, AND F. D. KING. 1976. Preservation of enzymic activity in marine phytoplankton by low temperature freezing. Mar. Chem. 4: 133–139.

ANSON, M. L. 1939. The estimation of pepsin, trypsin and cathepsin with haemoglobin. J. Gen. Physiol. 22: 79–89.

BOUCHER, J., A. Laurec, J. F. SAMAIN, AND S. L. SMITH. 1976. Etude de la nutrition, du régime et du rythme alimentaire du zooplancton dans les conditions naturelles, par la mesure des activités enzymatiques digestives, p. 85–110. Proc. 10th Europ. Mar. Biol. Symp. Vol. 2.

BOUCHER, J., AND J. F. SAMAIN. 1974. L'activité amylasique indice de la nutrition du zooplancton; mise en évidence d'un rythme quotidien en zone d'upwelling. Tethys 6: 179–188.

1975. Etude de la nutrition du zooplancton en zone d'upwelling par les mesures des activités enzymatiques digestives, p. 329–341. *In* H. Barnes [ed.] Proc. 9th Europ. Mar. Biol. Symp. Aberdeen University Press, Aberdeen.

CONOVER, R. J., AND P. MAYZAUD. 1976. Respiration and nitrogen excretion of neritic zooplankton in relation to potential food supply, p. 151–163. *In* G. Persoone and E. Jaspers [ed.] Proc. 10th Europ. Mar. Biol. Symp. Vol. 2. Universa Press, Wetteren, Belgium.

DAHLQVIST, A. 1964. Method for assay of intestinal disaccharidases. Anal. Biochem. 22: 99–107.

HESS, B., AND K. BRAND. 1974. Cell and tissue disintegration. Methods of enzymatic analysis, Vol. 1, p. 396–413. *In* H. V. Bergmeyer [ed.] Academic Press, New York and London.

KING, F. D., AND T. T. PACKARD. 1975. Respiration and the activity of the respiratory election transport system in marine zooplankton. Limnol. Oceanogr. 20: 849–853.

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MAYZAUD, P., AND R. J. CONOVER. 1976. Influence of potential food supply on the activity of digestive enzymes of neritic zooplankton, p. 415–427. *In* G. Personne and E. Jaspers [ed.] Proc. 10 Europ. Mar. Biol. Symp. Vol. 2. Universa Press, Wetteren, Belgium.

MAYZAUD, P., AND S. POULET. 1978. The importance of the time factor in the response of zooplankton to varying concentrations of naturally occurring particulate matter. Limnol. Oceanogr. 23: 1144–1154.

MORTON, R. K. 1955. Methods of extraction of enzymes from animal tissues, p. 25–51. *In* S. N. Colowick and N. O. Kaplan [ed.] Methods in enzymology. Vol. 1. Academic Press, New York and London.

PLATT, T. 1975. Analysis of the importance of spatial and temporal heterogeneity in the estimation of annual production by phytoplankton in a small, enriched, marine basin. J. Exp. Mar. Biol. Ecol. 18: 99–109.

PLATT, T., AND R. J. CONOVER. 1971. Variability and its effect on the 24h chlorophyll budget of a small marine basin. Mar. Biol. 10: 52–65.

PROSSER, C. L., AND P. B. VAN WEEL. 1958. Effect of diet on digestive enzymes in midgut gland of african giant snail, *Achatina fulica*. Physiol. Zool. 31: 171–178.

STREET, H. V., AND J. R. CLOSE. 1956. Determination of amylase activity in biological fluids. Clin. Chim. Acta 1: 256–268.

VACQUIER, V. D. 1971. The appearance of β-(1–3) glucanohydrolase activity during differentiation of the gut of sand dollar plutei. Dev. Biol. 26: 1–10.

VAN WEEL, P. B. 1970. Digestion in crustacea, p. 97–115. *In* M. Florkin and B. T. Scheer [ed.] Chemical zoology. Vol. 5. Academic Press, New York.

WORTHINGTON BIOCHEMICAL CO. 1972. Enzyme Manual. 216p.

Relationship Between Thermal Stability and Summer Oxygen Depletion in a Prairie Pothole Lake

M. H. PAPST, J. A. MATHIAS, AND J. BARICA

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

PAPST, M. H., J. A. MATHIAS, AND J. BARICA. 1980. Relationship between thermal stability and summer oxygen depletion in a prairie pothole lake. Can. J. Fish. Aquat. Sci. 37: 1433–1438.

Periods of summer oxygen depletion (summerkill), occurring in shallow prairie lakes, are dependent on the collapse of algae blooms but are not an obligatory result of the collapse. A period of thermal instability following this bloom collapse, or coincidental with it, is a necessary requirement. Wind stress and night-time air temperature are the principal factors determining the degree of thermal stability. These findings explain the speed with which oxygen depletion can occur, that the occurrence of algal biomass collapses without severe oxygen depletion (partial collapses), and the correlation between the occurrence of periods of lake oxygen depletion and changing weather conditions.

Key words: lake, summerkill, anoxia, mixing, oxygen depletion, thermal stability, weather, *Aphanizomenon*

PAPST, M. H., J. A. MATHIAS, AND J. BARICA. 1980. Relationship between thermal stability and summer oxygen depletion in a prairie pothole lake. Can. J. Fish. Aquat. Sci. 37: 1433–1438.

Les périodes d'épuisement d'oxygène en été (mortalités estivales) observées dans les lacs de prairie peu profonds dépendent des floraisons algales, mais n'en sont pas la conséquence obligatoire. Il faut une période d'instabilité thermique après, ou en même temps que l'affaissement de la floraison. La force du vent et la température de l'air la nuit sont les principaux facteurs responsables de la stabilité thermique. Ces découvertes expliquent la rapidité avec laquelle l'épuisement d'oxygène peut se produire, l'occurrence d'affaissements de la biomasse algale sans épuisement sérieux d'oxygène (affaissements partiels) et la corrélation entre l'occurrence de périodes d'épuisement d'oxygène d'un lac et des conditions météorologiques changeantes.

Received January 29, 1980
Accepted May 30, 1980

Reçu le 29 janvier 1980
Accepté le 30 mai 1980

THE shallow eutrophic lakes in the North American prairie can experience periods of oxygen depletion during the summer months (summerkill). These periods of oxygen depletion occur after the collapse of *Aphanizomenon flos-aquae* blooms (Barica 1975). Barica (1978) found that changes in weather conditions are associated with the occurrence of periods of lake oxygen depletion. One interpretation of this observed association between weather conditions and oxygen depletion is that the changes in weather conditions adversely affect the *A. flos-aquae* bloom, "triggering" the bloom's collapse (Barica 1978; Van Nguyen and Wood 1979). Such an interpretation implies that the sequence of events and order of causality is (1) deteriorating weather conditions (heavy overcast, with increased wind speeds, and decreasing air temperatures), (2) bloom collapse, (3) oxygen depletion. Further observations, however, do not support this interpretation. For example, declines in algal biomass sometimes precede deterioration of weather conditions by a day or two; oxygen depletion does not always follow after algal bloom collapse (Barica 1978); there may be a lag of several days, yet when it occurs the water column can undergo oxygen depletion in less than 6 h. Algal bloom collapse is not necessarily brought about by weather conditions, and oxygen depletion is not a simple and direct result of the bloom collapse.

Other mechanisms have been suggested to explain the occurrence of periods of oxygen depletion. Swingle (1968) reported that when the shallow thermal "stratification" of highly productive ponds broke down because of change in weather conditions, the resulting upwelling of anoxic bottom water resulted in oxygen depletion and fish kills. Although such an interpretation explains the observed association between weather conditions and periods of lake oxygen depletion, it does not explain the association of periods of oxygen depletion with algal bloom collapse. In shallow prairie lakes, thermal stability breaks down numerous times during the summer (Lakshman 1971; Sunde and Barica 1975), yet oxygen depletion is observed only infre-

Printed in Canada (J5950)
Imprimé au Canada (J5950)

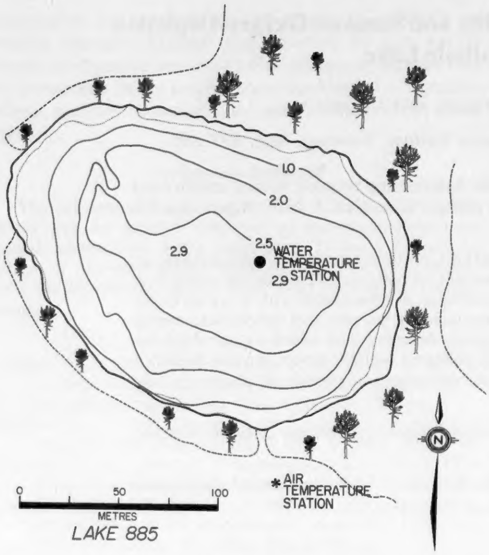


FIG. 1. Bathymetric chart of Lake 885. Maximum depth 2.9 m, area 2.4 ha, and mean depth 1.9 m.

quently. Thermal instability, then, would appear to be the only one of the variables determining the occurrence of periods of lake oxygen deficit.

We propose a mechanism in which both thermal instability and the algal bloom collapse act in concert to produce the observed oxygen depletion (summerkill) phenomenon. We suggest that a decline in algal biomass as well as a concomitant or subsequent period of thermal instability (mixing) are necessary conditions leading to lake oxygen depletion. Thus, oxygen depletion should not occur when (1) there has been no bloom collapse and there is thermal stability; (2) there has been no bloom collapse and there is thermal instability; (3) there has been a bloom collapse and there is thermal stability; and oxygen depletion should occur when there has been a bloom collapse and there is thermal instability. The objective of this study was to reexamine existing observations from prairie summerkill lakes to determine if our interpretation of the oxygen depletion phenomena was justified.

Methods

This study was conducted during 1977, and involved lake number 885 of the Central Canada Aquaculture study area (50°30′N–100°10′W). Lake 885 has been identified as a high summerkill risk lake (Barica 1975). The lake has no permanent inflow or outflow and is surrounded by trees, only exposed to the direct force of winds from the Northwest (Fig. 1).

Air temperatures were measured continuously using a Weksler recording thermometer mounted in a Stevenson screen located near the shore of the lake. Surface (0.25 m) and bottom water temperatures were continually recorded using Weksler thermometers mounted on a float over the deepest part of the lake. Laboratory tests indicated that the Weksler thermometers had a sensitivity of 0.2°C and a precision of ±0.2°C. Wind speed and direction were recorded using an Aerovane mounted on a metre tower at Erickson, Manitoba, 45 km from the lake. Wind speeds were expressed as kinematic wind stress ($m^2 \cdot s^{-2}$) and wind velocity ($m \cdot s^{-1}$). Daily means were calculated from hourly measurements of wind speed and direction; 2-h averages were also used for Fig. 5. Temperature profiles at 0.5-m intervals were periodically taken with YSI model 154 ABP oxygen and temperature meter. Oxygen concentrations were determined with the YSI model 154 or by the Winkler method (APHA 1971).

Chlorophyll A concentrations were determined by the fluorometric method (Stainton et al. 1974).

The difference between the mean daily surface and bottom lake temperatures was used as an index of lake thermal stability. Differences of ±1.0°C or more were termed stable conditions, differences of ±1.0°C to 0.0°C were termed neutral conditions, and conditions where the bottom temperature was greater than the surface temperature were termed unstable.

Primary production rates were measured in an incubator with a light gradient at 1-wk intervals from June 22 to August 11. The method is extensively described by Fee (1977) and details of the incubation technique are the same as those described by Shearer and Fee (1974). In situ rates were estimated with the numerical model. Both in situ and surface irradiances were measured with quantum sensors manufactured by Lambda Instruments Company.

Results

THERMAL INSTABILITY AND BLOOM COLLAPSE

The water column of Lake 885 was thermally neutral or unstable for five periods: A, (5 d); B, (5 d); C, (2 d); D, (1 d); and E, (4 d), between May 25 and August 2, 1977 (Fig. 2 (II)). Periods C and E were the only two periods, which were associated with severe water column oxygen depletion, i.e. whole oxygen concentration greater than 8.0 mg/L during period A (Fig. 3). The periods of severe oxygen deficit (C and E) were ~1 mo apart and the bottom metre of the water column had an oxygen concentration of <2.5 mg/L between these two periods (Fig. 3).

In accord with our interpretation of oxygen depletion, only periods C and E should have been associated with an algal bloom collapse. Algal bloom collapse has been defined by Barica (1978) as a decline in chlorophyll *a* concentration to about 20 $\mu g\ L^{-1}$, at a rate usually greater than 70 $\mu g\ L^{-1} \cdot wk^{-1}$. Of the five periods of neutral or unstable thermal condition during the summer of 1977 in Lake 885, periods C and E were preceded by periods of algal biomass decline with rates approaching 70 $\mu g \cdot L^{-1} \cdot wk^{-1}$ (Fig. 4). Prior to periods C and E the chlorophyll concentration of the lake fell to values of ~20 $\mu g\ L^{-1}$, indicative of an algal bloom collapse. Both periods are instances of bloom collapse with thermal stability. As shown in

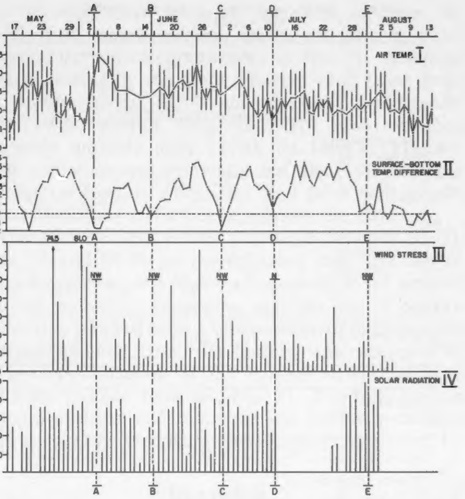


FIG. 2. Meteorological factors affecting lake thermal stability. I, mean daily air temperature, vertical bars indicate range; II, at surface, bottom water temperature; III, wind stress, as the square of wind speed; IV, total solar radiation.

Fig. 3, oxygen depletion occurred in both instances. Prior to period B, chlorophyll concentrations were declining rapidly but were still in the range of 70 to 100 $\mu g\ L^{-1}$ at the time of thermal instability. These conditions are an instance of thermal stability with no bloom collapse. Although no oxygen measurements were taken on June 15 (period B), the oxygen concentration remained high enough to support the rainbow trout (*Salmo gairdneri*) population throughout the period of instability, suggesting that oxygen concentrations were not less than 4–5 $mg \cdot L^{-1}$. There was one instance of a bloom collapse in which there was thermal instability during the summer. A rapid decrease (91.3 μg $chLa \cdot L^{-1}$) occurred between June 22 and June 23 (Fig. 4), resulting in a final concentration of 25.7 μg $chLa \cdot L^{-1}$ on June 23. This decrease corresponds to a rate of ~640 $\mu g\ chLa \cdot L^{-1} \cdot wk^{-1}$. However, the lake was thermally stable with a temperature difference, surface to bottom, of ~3°C, and no oxygen depletion occurred (Fig. 3).

Periods A and D (Fig. 4) are examples of thermal instability with no bloom collapse. During these periods, thermal instability occurred when the algal population was healthy and in a growth phase. Figure 3 shows that no oxygen depletion took place at these times.

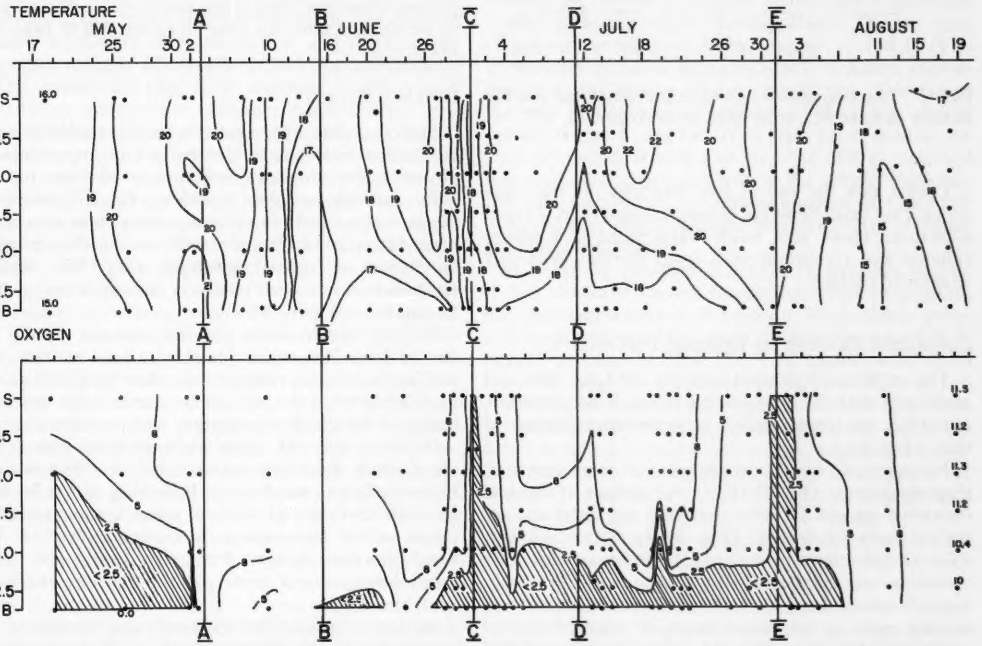


FIG. 3. Temperature and dissolved oxygen isopleths Lake 885, 1977. Temperature °C, dissolved oxygen mg/L.

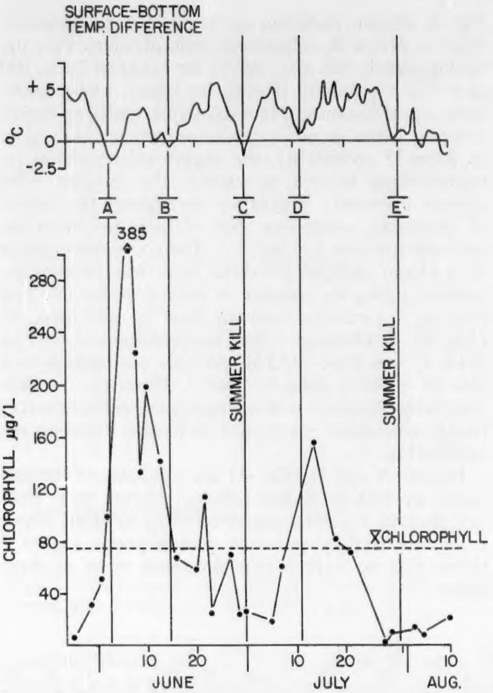


FIG. 4. Correspondence between chlorophyll and thermal stability in Lake 885, from May 20 to August 10, 1977.

Events near the end of July illustrate the time lags which can exist between bloom collapse and oxygen depletion. These lags result when there is a bloom collapse and conditions shift from thermal instability to thermal stability.

CONDITIONS LEADING TO THERMAL INSTABILITY

The erosion of thermal stability in Lake 885 was associated with increased wind stress, wind direction, nighttime air temperatures, or some combination of these (Fig. 2).

Periods A, C, and D are preceded or coincident with high wind stress (Fig. 2 (III)). All periods of thermal instability except C were preceded by nighttime air temperatures below 5°C (Fig. 2 (I), refer to lowest value of daily temperature range). Below-average solar radiation was associated with winds from the northwest direction. Lake 885 is surrounded by trees and is only open to the direct force of winds from the northwest direction (Fig. 1). The presence of this shelter belt around the lake undoubtedly affects the degree of turbulent mixing.

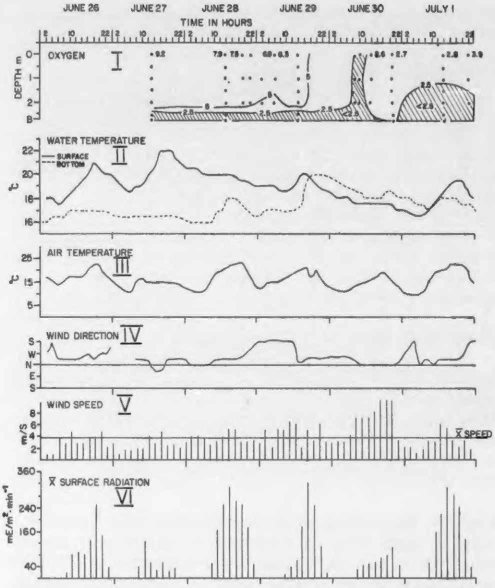


FIG. 5. Changes in oxygen, surface–bottom water temperature difference, wind speed, and surface radiation, prior to, during, and after, the June 30 summerkill in Lake 885, 1977.

DIURNAL EVENTS

Sufficient data were available so that period C could be studied in detail to determine the importance of diurnal meteorological conditions on lake thermal stability and lake oxygen depletion. The difference between surface and bottom temperature was at a maximum between 16:00 and 18:00 on the days prior to the period of thermal instability (Fig. 5B). Bottom temperatures remained relatively constant through June 26 and June 27 (Fig. 5B).

Surface temperatures did not increase during the day of June 28 in spite of increased air temperatures and increased solar radiation, perhaps because heat was transferred from the surface to deeper water layers as indicated by the rise in bottom water temperatures at ~10:00 on June 28. This was coincident with strong winds from the northwest, which is the direction for highest effective wind stress. Following sunset on June 28 both surface and bottom water temperatures declined as the air temperature declined (Fig. 5). The wind direction changed from north–northwest to the south, however, and the lake did not become thermally unstable.

Surface temperatures increased slightly during the morning and early afternoon of June 29 in response to increasing air temperature and solar radiation. The lake bottom water temperature began to rise following the shift in wind from south to northwest at noon. By 14:00 there was a marked rise in the bottom water temperature, which presumably is due to enhanced vertical mixing by the more effective wind stress from the northwest. By 18:00, the measured temperature profile was unstable (Fig. 4B). The lake remained thermally unstable until 12:00, on July 1 (Fig. 4). Solar radiation was markedly less than the seasonal average on June 30 (Fig. 5B), and there was no evidence of surface water heating during the day.

The lake became anoxic sometime between 20:00, June 29, and 08:00 on the following day. The rate of oxygen depletion was high and occurred at the time of thermal instability, suggesting that the loss of oxygen could have resulted from a resuspension of high-oxygen-demand material from the bottom water layer. The oxygen concentration of the bottom water had been <2.5 mg/L since June 26 (Fig. 3). The high oxygen demand of Lake 885 sediment and bottom water has been previously demonstrated (Barica 1974).

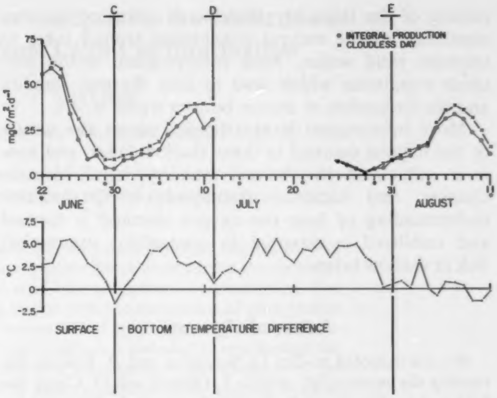


FIG. 6. Estimated primary production and thermal stability in Lake 885 from June 22 to August 11, 1977. *Sampling dates.

Discussion

Our observations indicate that the occurrence of lake oxygen depletion is dependent on the erosion of thermal stability at times coincident with or subsequent to a period of algal bloom collapse. Further, in the case of Lake 885 the trees surrounding the lake made wind direction a key factor in determining the wind stress actually applied to the lake surface and therefore an important variable affecting Lake 885 thermal stability.

The hypothesis that both thermal instability and algal bloom collapse are necessary conditions for oxygen depletion explains a number of otherwise conflicting observations. For example, algal bloom collapse did not result in lake oxygen depletion when the collapse was coincidental with a period of thermal stability; similarly, thermal instability did not lead to oxygen depletion when unaccompanied by a decline in algal biomass; finally, time lags between algal bloom collapse and oxygen depletion resulted when a period of thermal instability occurred sometime after the collapse of the bloom. The correlation between oxygen depletion and deteriorating weather conditions observed by Barica (1978) is valid. The explanation is that thermal instability of shallow prairie lakes often results from the occurrence of low pressure weather systems which are accompanied by high winds, low solar radiation, rain, and low air temperatures. Barica's (1978) observation that declining air temperature and solar radiation were usually, but not always, coincidental with periods of summerkill is explained by our observation that periods of thermal instability do not always result in oxygen depletion, since to do so they must occur during or after an algal bloom collapse.

Van Nguyen and Wood (1979) predicted that periods of low surface irradiance (cloudy days) trigger algal bloom collapse and oxygen depletion. Barica (1978) suggested that when high solar radiation is suddenly withdrawn (cloudy day) from a dense, but nutrient-deficient algal population, the bloom may collapse. Our primary production measurements, shown in Fig. 6, would support the prediction that algal primary production is nutrient limited rather than light limited at the time of the bloom collapse. The low rates of primary production during periods C and E (Fig. 6) were not increased when high solar radiation (cloudless day) was used to estimate production. This means that the photosynthetic capacity of the algae during periods C and E was low at all light levels used in the algal incubator. Healey and Hendzel (1976) reported that marked changes occurred in the cellular composition and metabolism of *Aphanizomenon* in Lake 885 as the blooms progressed and presented some evidence for nutrient deficiency.

It is usually assumed that oxygen depletion leading to fish deaths is caused by the increased metabolism and population growth of bacteria. While this is probably so in part, we have found the instantaneous (15 s) oxygen demand (APHA 1971) of anoxic water from near the lake bottom to be as high as $24.8\ \mathrm{mg\cdot L^{-1}}$ (unpublished data). This suggests that during periods of thermal stability when the bottom water layers become anoxic, a considerable oxygen debt can accumulate. Subsequent mixing would transfer this debt to the whole water column. Further research on this point in shallow lakes and fish ponds would be useful.

Attempts to manage small pothole lakes for extensive aquaculture have been limited by the risk of summerkill (Ayles et al. 1976). Efforts to control or predict summerkill have focused largely on the changes in algal biomass. The introduction of lake thermal stability as a key factor in determining summerkill risk offers an opportunity to consider other forms of management. Future research should investigate forced mixing of the lakes by mechanical means or the manipulation of the natural windbreaks around lakes to increase wind action. Such manipulation would preclude conditions which lead to lake thermal stability and the formation of anoxic bottom water layers.

More information is also needed about the nature of the oxygen demand in these shallow lakes, and how it is influenced by thermal stability, algal biomass changes, and bacterial decomposition. Quantitative understanding of how the oxygen demand is formed and stabilized is essential to controlling summerkill risk in shallow lakes.

Acknowledgments

We are indebted to Drs D. Schindler and B. Kenney for reading the manuscript, and to J. Gibson and G. Curry for field and analytical work. Special thanks to A. Furutani for aid with the primary production work.

AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION AMERICAN PUBLIC HEALTH ASSOCIATION, (APHA). 1971. Standard methods for the examination of water and wastewater. 13th ed. American Public Health Association, Washington, D.C. 874 p.

AYLES, G. B., AND J. BARICA. 1977. An empirical method for predicting trout survival in Canadian prairie lakes. Aquaculture 12: 181–185.

AYLES, G. B., J. G. I. LARK, J. BARICA, AND H. KLING. 1976. Seasonal mortality of rainbow trout (*Salmo gairdneri*) planted in small eutrophic lakes of central Canada. J. Fish. Res. Board Can. 33: 647–655.

BARICA, J. 1974. Extreme fluctuations in water quality of eutrophic fish kill lakes: effect of sediment mixing. Water Res. 8: 881–888.

1975. Collapse of algal blooms in prairie pothole lakes: their mechanisms and ecological impact. Int. Ver. Theor. Angew. Limnol. Verh. 19: 606–615.

1978. Collapses of *Aphanizomenon flos-aquae* blooms resulting in massive fish kills in eutrophic lakes: effect of weather. Int. Ver. Theor. Angew. Limnol. Verh. 20: 203–213.

FEE, E. J. 1977. A computer program for estimating annual primary production in vertically stratified water bodies with an incubator technique. Fish. Mar. Serv. Tech. Rep. 741: 38 p.

HEALEY, F. P., AND L. L. HENDZEL. 1976. Physiological changes during the course of blooms of *Aphanizomenon flos-aquae*. J. Fish. Res. Board Can. 33: 36–41.

LAKSHMAN, G. 1971. The water budget of shallow permanent or intermittent natural reservoirs as it affects economy and wildlife. Saskatchewan Research Council, Arda Program, Progress Rep. 6: 80 p.

SHEARER, J. S., AND E. J. FEE. 1974. Phytoplankton primary production in experimental lakes area using an incubator technique — 1973 data. Can. Fish. Mar. Serv. Res. Dev. Tech. Rep. 471: 110 p.

STAINTON, M. P., M. CAPEL, AND F. A. J. ARMSTRONG. 1974. The chemical analysis of fresh water. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

SUNDE, L., AND J. BARICA. 1975. Geography and lake morphometry of the aquaculture study area in the Erickson–Elphinstone district of southwestern Manitoba. Fish. Mar. Serv. Res. Dev. Tech. Rep. 510: 35 p.

SWINGLE, H. S. 1968. Fish kills caused by phytoplankton blooms and their prevention. Proc. World Symp. on Warm-water Pond Fish Culture. FAO Fish Rep. 44: 411 p.

VAN NGUYEN, V., AND E. F. WOOD. 1979. On the morphology of summer algae dynamics in non-stratified lakes. Ecol. Model. 6: 117–131.

A Procedure Using Modeling and Error Analysis for the Prediction of Lake Phosphorus Concentration from Land Use Information[1]

K. H. RECKHOW[2] AND J. T. SIMPSON[3]

Department of Resource Development, Michigan State University, East Lansing, MI 48824, USA

RECKHOW, K. H., AND J. T. SIMPSON. 1980. A procedure using modeling and error analysis for the prediction of lake phosphorus concentration from land use information. Can. J. Fish. Aquat. Sci. 37: 1439–1448.

Recent work in the area of empirical lake modeling and error analysis has resulted in some useful methods for the assessment of lake trophic quality. These methods are incorporated into the procedure presented herein for the prediction of lake phosphorus concentration. The procedure is described in a step-by-step manner; it begins with the estimation of phosphorus flux from major land use types and ends with the estimation of trophic state from the phosphorus concentration prediction. A nonparametric error analysis is included to supplement the prediction of phosphorus concentration. This assessment of uncertainty is an indication of the value of the information provided by the model. The method may be easily applied to most north temperate lakes, and an example is presented to illustrate its use.

Key words: eutrophication, lakes, model, error analysis, phosphorus, land use planning

RECKHOW, K. H., AND J. T. SIMPSON. 1980. A procedure using modeling and error analysis for the prediction of lake phosphorus concentration from land use information. Can. J. Fish. Aquat. Sci. 37: 1439–1448.

De récents travaux sur la modélisation empirique d'un lac et sur l'analyse de l'erreur ont conduit à d'utiles méthodes d'évaluation de son état trophique. Ces méthodes ont été incorporées dans le protocole décrit ici, qui permet de prédire la concentration de phosphore d'un lac. Nous présentons ce protocole étape par étape; la première étape consiste à estimer le flux de phosphore découlant des principaux types d'utilisation des terres et la dernière, à estimer l'état trophique à partir de la prévision de concentration de phosphore. À cette prévision, nous ajoutons une analyse d'erreur non paramétrique. Cette évaluation de l'incertitude permet de juger de la valeur de l'information fournie par le modèle. La méthode peut facilement s'appliquer à la plupart des lacs tempérés septentrionaux, et un exemple sert à illustrer sont utilisation.

Received August 27, 1979
Accepted May 28, 1980

Reçu le 27 août 1979
Accepté le 28 mai 1980

The Problem

AN increase in our wealth and leisure time has led to a greater demand for natural and recreational resources. Lakes, in particular, have become centers of population growth and intensified land use activity. Unfortunately, increased activity and development within the watershed of a lake almost always result in changes within the lake itself. These internal changes occur as a result of the introduction of sewage treatment plant effluent, septic tank leachate, lawn fertilizers, agricultural runoff, and other culturally induced "waste streams." Cultural inputs such as these represent the major sources of nitrogen and phosphorus, the two nutrients that, when in excess, may accelerate aquatic plant and algal growth and artificially "age" a lake (Vollenweider 1968).

Of these two nutrients, phosphorus is considered the most manageable. This is because (1) phosphorus is added primarily from allochthonous sources, that is, it originates outside the lake and, unlike nitrogen, it is not transported, in a gaseous state, across the air/water interface or fixed (i.e. transformed into a biologically usable form) by blue-green algae and bacteria; (2) in culturally impacted lakes, the proportion of the total supply of phosphorus directly attributable to man is typically higher than that for any other growth-limiting element (Vallentyne 1974); (3) phosphorus removal from waste water is technologically possible; and (4) nonpoint phosphorus pollution may be restricted with proper land use practices.

The quantitative and qualitative limits imposed by other growth factors such as nitrogen, carbon, and light are important, especially in enriched lakes. Because of limited manageability of these factors, however, relief from obnoxious aquatic plant and algal growth generally hinges on man's ability to reduce the amount of phosphorus available to the plants and algae (King 1979). Thus, watershed management aimed at

---

[1] Michigan AES journal article No. 9388.

[2] Present address (K.H.R.): School of Forestry and Environmental Studies, Duke University, Durham, NC 27706, U.S.A.

[3] Present address (J.T.S.): Department of Water Resources, 950 Leestown Road, Frankfort, KY 40601, USA.

Printed in Canada (J5758)
Imprimé au Canada (J5758)

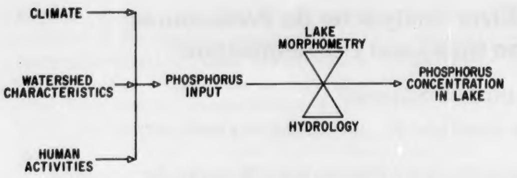


FIG. 1. Factors affecting lake phosphorus concentration.

limiting a lake's phosphorus supply (and hence the lake phosphorus concentration) is one of the few practical and permanent alternatives for the maintenance and improvement of our inland waters.

The Phosphorus Lake Model

To plan for the management of phosphorus in a lake watershed, mathematical models of lakes can be quite useful. Given the state of knowledge regarding phosphorus cycles, and the limited funds available to most planning agencies, often the most practical mathematical model for phosphorus management is the simple input/output or "black box" empirical model (Reckhow and Chapra 1979).

Figure 1 is a schematic of a "black box" model and represents the conceptual foundation for the mathematical model used in this procedure. The figure shows that phosphorus input (loading) to a lake is a result of climate, watershed characteristics, and human activities. This input is modified by environmental factors and yields an output: the lake's average phosphorus concentration.

The mathematical model proposed herein was developed by Reckhow (1979a) from 47 north temperate lakes included in the Environmental Protection Agency's National Eutrophication Survey. This model expresses phosphorus concentration (P, in mg/L) as a function of phosphorus loading (L, in g/m^2-yr), areal water loading (q_s, in m/yr), and apparent phosphorus settling velocity (v_s, in m/yr) in the form:

$$(1) \quad P = \frac{L}{v_s + q_s}$$

Using least squares regression, it was found that the apparent settling velocity could be fit using a weak function of q_s. This resulted in the fitted model:

$$(2) \quad P = \frac{L}{11.6 + 1.2q_s}.$$

A few limitations on the use of this model should be mentioned now. Since the model was constructed only from lakes within the north temperate climatic zone, it should be applied only to lakes within this zone. Furthermore, the model should not be applied to a lake with variable values more than the maximum values, or less than the minimum values, specified in Table 1. This is because an empirical model should not be used on lakes different from those used to develop the model, without prior testing. Finally, the model may be used to predict the average phosphorus concentration throughout a lake during the growing season. It cannot be used, as developed, to predict nearshore or short-term concentrations.

TABLE 1. Minimum and maximum values for the data set used to develop the phosphorus model (from Reckhow 1979a).

Variable	Minimum	Maximum
P	0.004 mg/L	0.135 mg/L
L	0.07 g/m^2-yr	31.4 g/m^2-yr
q_s	0.75 m/yr	187 m/yr

An important yet often overlooked aspect of the application of models is the fact that the model itself is a simplification of the real world, and thus the prediction from a model is inherently uncertain. Therefore, quantification of the prediction uncertainty should be a required step when a mathematical model is applied. This estimate of the prediction uncertainty could then be used by a modeler or planner as a weight indicating the value of the information contained in the prediction.

Uncertainty, or error, in this modeling exercise may arise from three primary sources: the model, the model parameters, and the model variables. Errors in the model and model parameters are derived from the procedure (regression analysis) used to fit empirically the model. For the phosphorus lake model (Equation 2), the model error ($s_{m\,\log}$) for the log transformed model is 0.128; parameter error was found to be quite small for most applications, so it was ignored. Model variables uncertainty is estimated for the application lake, and Steps 2 and 4 in the procedure presented herein illustrate the necessary calculations. The separate error terms are combined for an estimate of total prediction uncertainty in Step 4.

Once phosphorus concentration is predicted through the application of the empirical model, it is useful to interpret this prediction in the context of expected water quality characteristics in the lake of interest. One example of a trophic state ranking scheme or index was proposed by Chapra and Reckhow (1979) based on average phosphorus concentration. In an earlier work, Dillon and Rigler (1975) also devised a trophic classification scheme which related general water quality and lake use features to the traditional trophic states. These two indices are combined in Table 2 to link phosphorus concentration to potential lake use.

The Modeling Procedure

The method proposed herein has as its basis a paper presented in this journal a few years ago. The authors of that paper, Dillon and Rigler (1975), developed a procedure that may be used to calculate the capacity of a lake for development based upon the relationship be-

TABLE 2. Proposed relationships among phosphorus concentration, trophic state, and lake use. (Adapted from Chapra and Reckhow 1979; Dillon and Rigler 1975.)

Phosphorus concn. (mg/L)	Trophic state	Lake use
<0.010	Oligotrophic	Suitable for water-based recreation and propagation of cold-water fisheries, such as trout. Very high clarity and aesthetically pleasing.
0.010–0.020	Mesotrophic	Suitable for water-based recreation but not for cold-water fisheries. Clarity less than oligotrophic lakes.
0.020–0.050	Eutrophic	Limited total body contact suitability, based upon either loss of aesthetic properties or possible health hazards. Generally very productive for warmwater fisheries.
>0.050	Hypereutrophic	A typical "old aged" lake in advanced succession. Some fisheries, but high levels of sedimentation and algae or macrophyte growth may be diminishing open water surface area.

tween phosphorus input and water quality. Our contribution, beyond that of Dillon and Rigler, lies in two areas. First, the most important improvement is the addition of an error estimation procedure. This permits the quantification of prediction uncertainty, and it indicates to the user how valuable (certain) the information is that is provided by the model. Second, the phosphorus lake model imbedded in this procedure has a wider range of applicability than does the Dillon–Rigler model. Dillon and Rigler derived their model from a highly homogeneous set of lakes; the model development data set for the model presented herein includes a fairly wide range of lake types (see Table 1).

To facilitate understanding of the impact assessment procedure, it is presented in conjunction with an application to Higgins Lake in Michigan. It should not be inferred from this that the procedure, as presented, is applicable only to Higgins Lake. On the contrary, the procedure is quite general and can be easily applied to most lakes (subject to north temperate location and data set constraints such as those in Table 1) simply by subsituting the appropriate lake data for the Higgins Lake data in the example. A more detailed account of this procedure, along with a thorough study of nutrient export coefficients, is presented elsewhere (Reckhow et al. 1980).

Higgins Lake, located in the northern, lower peninsula of Michigan, is a deep, cool, oligotrophic lake with a well-oxygenated hypolimnion. The lake has a maximum depth of 41 m and a mean depth of 15 m. Land use in the watershed is primarily rural residential or recreational. Some agricultural activity occurs in the watershed but most of the area is forested. Figure 2 identifies Higgins Lake, its watershed boundaries, and existing land uses.

For the sake of this example, let us assume that an estimate of average lake phosphorus concentration in Higgins Lake, along with an assessment of water quality and recreational potential, is needed for a planning study. A measure of prediction uncertainty is also needed to evaluate the results and to compare them with alternative studies.

The method and model presented above will be used to solve this problem. Recall that the model is

$$P = \frac{L}{11.6 + 1.2q_s}.$$

This analysis is structured so that the variables are estimated in the following order: Step 1, areal water loading (q_s); Step 2, areal phosphorus loading (L); Step 3, lake phosphorus concentration (P); and Step 4, phosphorus prediction uncertainty (s_T).

STEP 1: ESTIMATION OF q_s (AREAL WATER LOADING)

The estimation of q_s involves the solution of two equations:

$$(3)\quad Q = (A_d \times r) + (A_0 \times Pr)$$

$$(4)\quad q_s = \frac{Q}{A_0}$$

where:

q_s = areal water loading (m/yr)
Q = inflow water volume to lake (10^6 m^3/yr)
A_d = watershed area (10^6 m^2)
A_0 = lake surface area (10^6 m^2)
r = total annual unit runoff (m/yr)
Pr = mean annual net precipitation (m/yr).

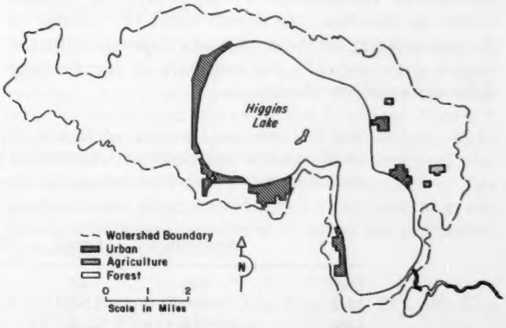


FIG. 2. Existing land use in the Higgins Lake watershed (adopted from U.S.E.P.A. 1975 and Liebeskind et al. unpublished data).

TABLE 3. Higgins Lake data necessary for the estimation of q_s.

Variable	Estimate
A_d = Watershed area	87.41×10^6 m^2
r = Total annual unit runoff	0.2415 m/yr
A_0 = Lake surface area	38.4×10^6 m^2
Pr = Mean annual net precipitation	0.254 m/yr

Ideally, Q should be determined from direct measurement of inflow or outflow, since use of any equation like Equation 3 will result in uncertainty in the predicted variable. When data for Q are not available, it becomes necessary to estimate A_d, A_0, r, and Pr and substitute them into Equation 3 to find Q.

The statistics required to solve Equations 3 and 4 for the Higgins Lake example are presented in Table 3. Therefore, the necessary variables are

a) Total annual inflow volume of water to Higgins Lake:

$$Q = (A_d \times r) + (A_0 \times Pr)$$
$$= 30.863 \times 10^6 \text{ m}^3\text{/yr.}$$

b) The areal water loading:

$$q_s = \frac{Q}{A_0}$$
$$= 0.804 \text{ m/yr.}$$

STEP 2: ESTIMATION OF L (AREAL PHOSPHORUS LOADING

Every watershed has a unique pattern of land use within its boundaries. Each land use contributes (by way of overland runoff or groundwater transport) to the phosphorus loading to a lake. For example, one common source of phosphorus is the shoreline septic tank-tile field system. Factors that determine the phosphorus loading from this source include (1) per capita phosphorus contribution to septic systems located within an identified lake impact zone; (2) number of persons utilizing this form of waste disposal within the impact zone; and (3) the capability of the drainage fields to immobilize phosphorus.

The total annual mass flow of phosphorus to a lake is estimated by summing the annual phosphorus contribution from each of the nonpoint sources plus any additional point source input within the watershed. Total mass loading (M) may be expressed as (in kg/yr):

$$(5) \quad M = (Ec_f \times \text{area}_f) + (Ec_{ag} \times \text{area}_{ag}) + (Ec_u \times \text{area}_u) + (Ec_p \times A_0) + (Ec_{st} \times \text{No. of capita-years} \times (1 - \text{S.R.})) + \text{PSI}$$

where:

Ec_f = export coefficient for forest land (kg/10^6 m^2-yr)
Ec_{ag} = export coefficient for agricultural land (kg/10^6 m^2-yr)
Ec_u = export coefficient for urban area (kg/10^6 m^2-yr)
Ec_p = export coefficient for precipitation (kg/10^6 m^2-yr)
Ec_{st} = export coefficient to septic tank systems impacting the lake (kg/(capita-yr) − yr)
Area$_f$ = area of forest land (10^6 m^2)
Area$_{ag}$ = area of agricultural land (10^6 m^2)
Area$_u$ = area of urban land (10^6 m^2)
A_0 = area of lake (10^6 m^2)
No of capita-years = No of capita years in watershed serviced by septic tank/tile field systems impacting the lake
S.R. = Soil retention coefficient (dimensionless)
PSI = Point source input (kg/yr).

General nonpoint phosphorus source categories are identified in Table 4 along with a range of phosphorus export coefficients. An export coefficient represents the expected annual amount of phosphorus transported, per unit of source (e.g. per square metre of farmland), to a surface water body. This table is representative of the export coefficients found in the literature (e.g. Uttormark et al. 1974) and has been classified by the authors into high, low, and midrange estimates. Thus, the assignment of an export coefficient to a particular category should be dependent on conditions present in the watershed of study. A range of phosphorus export coefficients is presented because the broadness of the "source categories" leaves room for much variation, due to basin geology, erosional patterns, and intensity and types of use.

TABLE 4. Phosphorus export coefficients (units are kg/10^6 m^2-yr, except septic tank as indicated; values are adapted from Uttormark et al. 1974 and Reckhow et al. 1980).

	Agriculture	Forest	Precipitation	Urban	Input to septic tank (kg/capita-yr)
High	300	45	60	500	1.8
Mid	40–170	15–30	20–50	80–300	0.4–0.9[a]
Low	10	2	15	50	0.3

[a] The value selected will depend, in part, upon whether or not phosphate detergents are permitted.

In practical applications it is recommended that high, most likely, and low export coefficients be selected for some of the phosphorus source categories. This allows the calculation of high, most likely, and low total loading estimates. The high and low loading estimates represent the additional phosphorus loading error that must be added to the model error for the calculation of total prediction uncertainty. It is important that the high and low loadings represent only those characteristics described in the steps below. This is because to a great extent, the error in the phosphorus loading estimates is already contained in the model error. Additional loading error for an application lake must be included only when the loading is estimated (using the procedure herein) in a different (and less precise) manner than it was estimated for the model development data set. These differences are described in the following steps.

The selection of appropriate phosphorus export coefficients is a difficult task. Since a critical aspect of this modeling exercise is the estimation of prediction errors, the analyst should realize that poor choice of export values contributes to an increase in error. This contribution may be explicit or implicit in the analysis, depending upon whether or not the analyst is aware of all of the uncertainty introduced by his/her choice of phosphorus export coefficients. Clearly, experience in the application of this modeling approach is a valuable attribute.

The procedure is presented below by way of example. This was done so that the mechanics of the calculations, particularly the error analysis in Step 4, could be easily understood. In this example, however, we have not explained our analyses or thought processes involved in the choice of phosphorus export coefficients. This information is application-specific, and may not be understood without considerable discussion of Higgins Lake and its watershed. In the interests of a concise presentation of this technique, it was not included. However, when this procedure is used to assist lake quality management planning, we strongly urge that the analyst present, in the report, all information used in the selection of export coefficients. This facilitates good planning. In addition, while Table 4 contains a summary of phosphorus export coefficients, we suggest that the analyst consult Uttormark et al. (1974) or Reckhow et al. (1980) for details on the conditions under which these literature values were generated. Proper choice of export coefficients is a function of knowledge of the application lake watershed and knowledge of the watersheds of candidate export coefficients. It is through comparisons of these watersheds that the analyst arrives at the appropriate coefficients.

The estimates of phosphorus loading error are based on high, most likely, and low phosphorus export coefficients selected by the analyst. Loading uncertainty may be caused by either variability or bias. Variability may result from natural fluctuations inherent in a characteristic (e.g. natural variations in streamflow or stream phosphorus concentration), or from uncertainty inherent in a statistic summarizing a set of data. Bias may result from a number of causes, all associated with the fact that the estimate may not be representative of the characteristic that it was selected to estimate. For example, some uncertainty due to possible bias is appropriate to situations where phosphorus export coefficients generated in one watershed are applied in another watershed. As the analyst becomes more uneasy about a selected export coefficient, he/she should express this uneasiness through increased uncertainty and a greater range between high and low export coefficients.

At different points in the procedure presented below, the analyst is alerted to possible sources of bias. These result from the difference between conditions in the model development data set lakes and application lakes. When the characteristics of these two lake groups differ substantially, it is possible that a procedure appropriate for analysis of one group is inappropriate for analysis of the other. This is the justification for Table 1, which presents the limitations on the basic model variables, as defined by the model development data set. Now, when the allocation of phosphorus loading sources differs between the two lake groups, there is probably no need to restrict the use of the model, which is insensitive to the source of phosphorus. However, the error analysis (but not the mean prediction) may be affected because the loading estimation errors vary from source to source. Therefore, warnings of possible bias are stated herein when the application lake phosphorus loading allocation differs substantially from that for the model development data set. These warnings should be addressed, when appropriate, by the inclusion of a bias uncertainty addition to the high and/or low loading estimates.

To facilitate the understanding and estimation of the variables contained in Equation 5, Step 2 has been broken into seven substeps.

STEP 2A: ESTIMATION OF AREA$_f$, AREA$_{ag}$, AND AREA$_u$ (WATERSHED AREAS)

Recall that the watershed area was determined in Step 1. This area must now be subdivided into agricultural, forest, and urban lands. The area for each must be determined and expressed in 10^6 m^2. Figure 2 identifies the existing land uses in the Higgins Lake watershed. The land use areas were determined and are presented in Table 5. When the objective of this analysis is the projection of future conditions, high and low area estimates are needed to reflect the uncertainty in the land use projections.

STEP 2B: ESTIMATION OF Ec_f, Ec_{ag}, Ec_u, Ec_p, AND Ec_{st} (EXPORT COEFFICIENTS)

This substep requires that an export coefficient (Ec_x) be chosen for each of the phosphorus source categories

TABLE 5. Land use areas in the Higgins Lake watershed (A. Liebeskind et al., Department of Resource Development, Michigan State Univ., East Lansing, MI, 1978, unpublished data).

Land use	Area (10^6 m^2)
Agriculture	0.16
Forest	83.47
Urban	3.78

listed in Table 4. The high and low export coefficients selected should reflect the modeler's confidence in the extrapolation of literature export values to the application lake watershed. For example, in cases where the modeler knows that the "most likely" export coefficient chosen was determined under a good sampling program on a watershed quite similar to the application lake watershed, the high and low values should be selected to represent little uncertainty. A single precipitation loading figure (the "most likely" value) is probably adequate for all conditions. Only when the precipitation loading is deemed substantial (perhaps 25% of the total loading) should it be necessary to include possible precipitation loading error bias.

When the objective is future projection, the area estimates and export coefficients should be combined according to: high area with high export, most likely area with most likely export, and low area with low export. This calculation is to be made in Step 2F.

Export coefficients were selected by the authors to describe conditions within the Higgins Lake watershed and are presented in Table 6. Note that high and low values are not the same as the high and low range values found in Table 4. This is because local conditions were judged to be not equivalent to the "extreme" conditions that are represented by the range in the table.

STEP 2C: ESTIMATION OF NO. OF CAPITA-YEARS

The number of persons contributing to septic systems that impact the lake must be estimated and expressed in capita-years. In this particular example it was assumed that only septic systems of lakeside dwellings impact Higgins Lake. When deemed appropriate, one should also include the phosphorus loading of systems that border tributaries with direct surface water contact to the lake. In some instances the user may desire to include a wider impact strip of land around the lake because of drainage patterns, shallow water tables, land slope, etc. It should be noted that Ellis and Childs (1973) demonstrated that groundwater can transport significant amounts of phosphorus from septic systems greater than 33 m. In addition, poorly drained tile fields within the entire watershed may be another phosphorus source. Clearly, the uniqueness of each watershed necessitates a thorough analysis so that the septic tank contribution of total phosphorus can be estimated.

When the model is used to assess current conditions, population surveys are quite useful for the estimation of the phosphorus loading from septic tanks. When the goal is the prediction of future conditions, population projections must be consulted. For most lakes, the high and low loading estimates for septic systems should then be based solely on the uncertainty in the population projections (the source of possible bias). The total number of capita-years may be calculated by adding together permanent resident capita-years and seasonal resident capita-years. This is described in Equation 6 below.

$$
\begin{aligned}
&\text{Total no. of capita-years} = \\
&\qquad\qquad \text{Permanent capita-year} \\
&\begin{matrix}\text{average no. of persons}\\ \text{per living unit}\end{matrix} \times \frac{\begin{matrix}\text{no. days spent}\\ \text{at unit per year}\end{matrix}}{365} \times \begin{matrix}\text{no. of}\\ \text{living units}\end{matrix} \\
(6)\qquad &\qquad\qquad + \\
&\qquad\qquad \text{Seasonal capita-year} \\
&\begin{matrix}\text{average no. of persons}\\ \text{per living unit}\end{matrix} \times \frac{\begin{matrix}\text{no. days spent}\\ \text{at unit per year}\end{matrix}}{365} \times \begin{matrix}\text{no. of}\\ \text{living units}\end{matrix}
\end{aligned}
$$

According to the EPA National Eutrophication Survey (U.S.E.P.A. 1975) there are an estimated 1000 seasonal dwellings on the Higgins Lake shoreline and all are served by septic systems. A facilities plan study estimated that each seasonal unit is occupied by an average of 3.5 people, who spend 60 d a year at their residence (Progressive Engineering Consultants 1976). This information may be inserted in Equation 6 to estimate the number of capita-years impacting the lake.

$$
\begin{aligned}
& \qquad\quad \text{Permanent} \qquad\qquad \text{Seasonal} \\
\text{Total no. of capita-years} &= \quad 0 \quad + 3.5 \times \frac{60}{365} \times 1000 \\
&= 575.3
\end{aligned}
$$

TABLE 6. High, most likely, and low export coefficients selected for Higgins Lake.

Source	High	Most Likely	Low
Forest	40 kg/10^6 m^2-yr	20 kg/10^6 m^2-yr	2 kg/10^6 m^2-yr
Agriculture	80 kg/10^6 m^2-yr	30 kg/10^6 m^2-yr	10 kg/10^6 m^2-yr
Urban	150 kg/10^6 m^2-yr	90 kg/10^6 m^2-yr	50 kg/10^6 m^2-yr
Precipitation	50 kg/10^6 m^2-yr	30 kg/10^6 m^2-yr	15 kg/10^6 m^2-yr
Input to Septic Tanks	1.0 kg/capita-yr	0.6 kg/capita-yr	0.3 kg/capita-yr

STEP 2D: ESTIMATION OF S.R. (SOIL RETENTION COEFFICIENT)

On-site septic tank–tile field systems may or may not be effective in trapping phosphorus and preventing it from entering a lake via groundwater transport. The soil retention coefficient is an estimate of how well the systems immobilize phosphorus. This coefficient may range from 0 to 1.0. For example, if it is assumed that all phosphorus transported to septic systems eventually reaches the lake, then a soil retention coeffcient value of 0 would be selected. If it is assumed that no phosphorus reaches the lake, then S.R. = 1.0.

There are four general mechanisms of phosphate removal in soils: (1) rapid removal or sorption; (2) slow mineralization and insolubilization; (3) plant uptake; and (4) biological immobilization (Tofflemire and Chen 1977). The most important phosphorus immobilization mechanisms in septic systems are the formation of insoluble iron and aluminum phosphate compounds and the adsorption of phosphate ions onto clay lattice structures (Tilstra et al. 1972).

Rodiek (1979) notes that effective tile drainage fields involve both physical and chemical processes. Chemical fixation reactions require effluent-to-soil contact of sufficient time length for chemical reactions or adsorption to occur. Subsurface permeability, slope, and water table depth are all factors influencing contact duration. When estimating S.R., consideration should be given to the age of the systems being surveyed, since a given volume of soil only has a limited phosphorus adsorption capacity.

When the model is used to predict future conditions, it is often sufficient to use a single estimated soil retention coefficient. Only when the estimated loading from septic systems is thought to be substantial (perhaps 25% of the total loading), should it be necessary to employ low, most likely, and high soil retention coefficients. This situation may occur (according to the U.S.E.P.A. (1975), but not according to this analysis) for Higgins Lake, so three retention coefficients are used in this example. It is possible, however, that the error analysis may be biased when the septic tank loading becomes a sizeable fraction of the total loading.

A "most likely" S.R. coefficient of 0.75, a "low" coefficient of 0.95, and a "high" coefficient of 0.50 were selected to represent the soils surrounding Higgins Lake.

STEP 2E: ESTIMATION OF PSI (POINT SOURCE INPUT)

If the effluent from an industry, sewage treatment facility, or other point source is deposited within the watershed, the impact must be assessed and expressed in kg phosphorus/yr.

At the present time there are no known point sources of phosphorus in the Higgins Lake watershed. Thus, PSI = 0 kg/yr. However, if point sources exist in the application lake watershed, or are projected for the future, then the uncertainty in the phosphorus loading from this source is represented by the uncertainty in the size of the projected population to be served.

As a final note on the phosphorus loading sources, it is possible that the lake sediments may be a nonnegligible source. If this is thought to be so for an application lake, then high, most likely, and low loading estimates should be used for the prediction and prediction error (see Reckhow 1979b for suggested values), to reflect this source of possible bias in the uncertainty analysis.

STEP 2F: CALCULATION OF M (TOTAL PHOSPHORUS MASS LOADING)

When Steps 2A through 2E are complete, Equation 5 may be solved to yield high, most likely, and low phosphorus mass loading estimates based on high, most likely, and low phosphorus export and soil retention coefficients.

Thus, for the Higgins Lake example:

High

$$\begin{aligned} M_{(\text{high})} &= (40 \times 83.47) + (80 \times 0.16) + (150 \times 3.78) + \\ &\quad (50 \times 38.4) + (1.0 \times 575.3 \times (1 - 0.5)) + 0 \\ &= 6126.3 \text{ kg/yr.} \end{aligned}$$

Most likely (ml)

$$\begin{aligned} M_{(\text{ml})} &= (20 \times 83.47) + (30 \times 0.16) + (90 \times 3.78) + \\ &\quad (30 \times 38.4) + (0.6 \times 575.3 \times (1 - 0.75)) + 0 \\ &= 3252.7 \text{ kg/yr.} \end{aligned}$$

Low

$$\begin{aligned} M_{(\text{low})} &= (2 \times 83.47) + (10 \times 0.16) + (50 \times 3.78) + \\ &\quad (15 \times 38.4) + (0.3 \times 575.3 \times (1 - 0.95)) + 0 \\ &= 942.2 \text{ kg/yr.} \end{aligned}$$

STEP 2G: CALCULATION OF L (ANNUAL AREAL PHOSPHORUS LOADING)

To be used in the model, annual phosphorus input must be expressed as a loading per unit lake surface area. This is accomplished by dividing M by the lake surface area, A_0:

$$L = \frac{M}{A_0}. \tag{7}$$

The units are then converted so that this areal phosphorus loading term is expressed in grams per square metre of lake surface area per year. Thus, for Higgins Lake.

High

$$\begin{aligned} L_{(\text{high})} &= \frac{6126.3 \text{ kg/yr}}{38.4 \times 10^6 \text{ m}^2} \\ &= 160 \times 10^{-6} \text{ kg/m}^2\text{-yr} = 0.160 \text{ g/m}^2\text{-yr} \end{aligned}$$

Most likely (ml)

$$\begin{aligned} L_{(\text{ml})} &= \frac{3252.7 \text{ kg/yr}}{38.4 \times 10^6 \text{m}^2} \\ &= 85 \times 10^{-6} \text{ kg/m}^2\text{-yr} = 0.085 \text{ g/m}^2\text{-yr.} \end{aligned}$$

Low

$$L_{(\text{low})} = \frac{942.2 \text{ kg/yr}}{38.4 \times 10^6 \text{ m}^2}$$
$$= 25 \times 10^{-6} \text{ kg/m}^2\text{-yr} = 0.025 \text{ g/m}^2\text{-yr}.$$

STEP 3: CALCULATION OF P (LAKE PHOSPHORUS CONCENTRATION)

The model may now be solved for high, most likely, and low phosphorus concentrations by substituting in values of q_s and L (high, ml, and low).

$$P_{(\text{high})} = \frac{L_{(\text{high})}}{11.6 + 1.2q_s}$$

$$P_{(\text{ml})} = \frac{L_{(\text{ml})}}{11.6 + 1.2q_s}$$

$$P_{(\text{low})} = \frac{L_{(\text{low})}}{11.6 + 1.2q_s}$$

For Higgins Lake:

High

$$P_{(\text{high})} = \frac{0.160}{11.6 + 1.2\,(0.804)} = 0.0127 \text{ mg/L}.$$

Most likely

$$P_{(\text{ml})} = \frac{0.085}{11.6 + 1.2\,(0.804)} = 0.0068 \text{ mg/L}.$$

Low

$$P_{(\text{low})} = \frac{0.025}{11.6 + 1.2\,(0.804)} = 0.0020 \text{ mg/L}.$$

STEP 4: ESTIMATION OF s_T (PREDICTION UNCERTAINTY)

To estimate the uncertainty associated with a prediction calculated using the phosphorus model, estimates are needed for the error, or uncertainty, in all terms in the model, and in the model itself. However, it has been shown by Reckhow (1979a) that for most applications of this model, the error in the parameter v_s is small. Further, error in q_s is primarily a function of flow measurement error and hydrologic variability, which also affect L. Since L and q_s are in the numerator and denominator, respectively, in the model, the errors affecting both tend to cancel when they are combined to yield the resultant error in P. In addition, hydrologic variability is unimportant in lakes with low flushing rates. Therefore, it is assumed here that the prediction error is a function only of model error and of aspects of phosphorus loading uncertainty that are identified in step 2. If the application lake flushes rapidly and is subject to great variations in year-to-year precipitation, then the modeler is urged to include hydrologic variation in the error analysis using the error propagation equation (Reckhow 1979a).

The model error is represented by $s_{m\log}$ in the equations below and is expressed in logarithmic units of phosphorus concentration error. The loading error, s_L, on the other hand, is expressed in untransformed units of phosphorus loading error. Therefore, to combine these two values for an estimate of total prediction uncertainty, some calculations are necessary.

The procedure presented below is based on first-order error analysis (Benjamin and Cornell 1970). In this particular application, three assumptions are of some importance:

1) Model error, expressed in log-transformed concentration units, is appropriately combined with variable error terms after the transformation is removed.
2) The "range" ("high" minus "low"), for phosphorus loading error, is about two times the standard deviation. This is based loosely on the characteristics of the Chebyshev inequality identified below, where about 90% of the distribution is contained within ±2 SD of the mean.
3) The individual error components are adequately described by their variances (or by their SD).

To relax a previously imposed (Reckhow 1979a) yet tenuous normality assumption, the confidence intervals constructed below are based on a modification of the Chebyshev inequality (Benjamin and Cornell 1970). Therefore, it is no longer required that the total error term be normally distributed. Instead, its distribution must only be unimodal and have "high order contact" with the abscissa in the distribution tails. These are achievable assumptions under almost all conditions, and it is recommended (Reckhow and Chapra 1979) that this type of nonparametric approach be adopted until the distributions have been adequately studied and characterized.

STEP 4A: CALCULATION OF LOG $P_{(\text{ml})}$

Take the logarithm of the most likely phosphorus concentration, $P_{(\text{ml})}$.

For Higgins Lake:

$$\log P_{(\text{ml})} = \log 0.0068$$
$$= -2.167$$

STEP 4B: ESTIMATION OF s_m+ ("POSITIVE" MODEL ERROR)

The model error, ($s_{m\log}$), was determined to be 0.128. Add $s_{m\log}$ to log $P_{(\text{ml})}$ and take the antilog of this value. Now calculate the difference between this antilog value and $P_{(\text{ml})}$. Label this difference s_m+, it represents the "positive" model error.

$$s_m+ = \text{antilog}\,[\log P_{(\text{ml})} + s_{m\log}] - P_{(\text{ml})} \tag{8}$$

For Higgins Lake:

$$s_m+ = \text{antilog}\,(-2.167 + 0.128) - 0.0068$$
$$= 0.0023 \text{ mg/L}.$$

Step 4C: Estimation of s_m- (“Negative” Model Error)

Subtract $s_{m\log}$ from log $P_{(\mathrm{ml})}$ and take the antilog of this value. Now calculate the difference between this antilog and $P_{(\mathrm{ml})}$, and label this difference s_m-.

(9) $s_m- = \text{antilog}\,[\log P_{(\mathrm{ml})} - s_{m\log}] - P_{(\mathrm{ml})}$

For Higgins Lake:

$$\begin{aligned} s_m- &= \text{antilog}\,(-2.167 - 0.128) - 0.0068 \\ &= -0.0017 \text{ mg/L.} \end{aligned}$$

Step 4D: Estimation of s_L+ (“Positive” Loading Error)

Now, one must convert the loading error estimate into units compatible with the model error. Use the $P_{(\mathrm{high})}$ concentration estimated in step 3 and calculate the difference between $P_{(\mathrm{high})}$ and $P_{(\mathrm{ml})}$; then divide this difference by 2. Label this value s_L+; it represents the “positive” loading error contribution.

(10) $s_L+ = \dfrac{P_{(\mathrm{high})} - P_{(\mathrm{ml})}}{2}$

For Higgins Lake:

$s_L+ = 0.0030$ mg/L.

Step 4E: Estimation of s_L- (“Negative” Loading Error)

Repeat step 4D substituting the low concentration value $P_{(\mathrm{low})}$ for $P_{(\mathrm{high})}$. Label the resultant value s_L-; it represents the “negative” loading error contribution.

(11) $s_L- = \dfrac{P_{(\mathrm{ml})} - P_{(\mathrm{low})}}{2}.$

For Higgins Lake:

$s_L- = 0.0024$ mg/L.

Step 4F: Estimation of s_T+ (Total “Positive” Uncertainty)

Total positive prediction uncertainty is calculated using the equation:

(12) $(s_T+)^2 = (s_m+)^2 + (s_L+)^2$

or

(13) $s_T+ = \sqrt{(s_m+)^2 + (s_L+)^2}$

For Higgins Lake:

$$\begin{aligned} s_T+ &= \sqrt{(0.0023)^2 + (0.0030)^2} \\ &= 0.0038 \text{ mg/L.} \end{aligned}$$

Step 4G: Estimation of s_T- (Total “Negative” Uncertainty)

Total negative prediction uncertainty is calculated using the equation:

14) $(s_T-)^2 = (s_m-)^2 + (s_L-)^2$

or

(15) $s_T- = \sqrt{(s_m-)^2 + (s_L-)^2}$

For Higgins Lake:

$$\begin{aligned} s_T- &= \sqrt{(-0.0017)^2 + (0.0024)^2} \\ &= 0.0029 \text{ mg/L.} \end{aligned}$$

Step 4H: Calculation of Confidence Limits

The prediction uncertainty may be expressed in terms of “confidence limits” which represent the prediction plus or minus the prediction uncertainty. Confidence limits have a definite meaning in classical statistical inference; they define a region in which the true value will lie a prespecified percentage of the time.

Using the modification of the Chebyshev inequality (Benjamin and Cornell 1970), the confidence limits may be written as:

(16) $\text{Prob}\,[(P_{(\mathrm{ml})} - hs_T-) \le P \le (P_{(\mathrm{ml})} + hs_T+)] \ge 1 - \dfrac{1}{2.25\,h^2}$

Equation 16 states that the probability that the true phosphorus concentration lies within certain bounds, defined by a multiple, h, of the prediction error, is greater than or equal to $1-1/2.25\,h^2$. (This relationship loses its significance as h drops much below one.) Substituting values for h into Equation 16 reveals that a value of one for h corresponds to a probability of about 55% (0.556 to be exact), and a value of two for h corresponds to a probability of about 90% (0.889 to be exact). Thus the 55% confidence limits are

(17) $\text{Prob}\,[(P_{(\mathrm{ml})} - s_T-) \le P \le (P_{(\mathrm{ml})} + s_T+)] \ge 0.55$

Substituting the Higgins Lake data this becomes:

$$\text{Prob}\,[(0.0068 - 0.0029) \le P \le (0.0068 + 0.0038)] \ge 0.55$$

$$\text{Prob}\,[0.0039 \text{ mg/L} \le P \le 0.0106 \text{ mg/L}] \ge 0.55.$$

Now that specific values for the prediction error have been inserted into the confidence limits expression, its interpretation changes somewhat. It is “about 55% of the time (that confidence limits are estimated), one can expect that the actual phosphorus concentration will lie within the bounds defined by the prediction plus or minus the prediction uncertainty.” This same interpretation format applies when the confidence limits are widened to the 90% level ($h = 2$), and the Higgins Lake data are inserted

(18) $\text{Prob}\,[(P_{(\mathrm{ml})} - 2s_T-) \le P \le (P_{(\mathrm{ml})} + 2s_T+)] \ge 0.90$

Inserting the data yields

$$\begin{aligned} \text{Prob}\,[((0.0068) - (2)\,(0.0029)) &\le P \\ &\le (0.0068 + (2)\,(0.0038))] \ge 0.90. \end{aligned}$$

$$\text{Prob}\,[0.0010 \text{ mg/L} \le P \le 0.0144 \text{ mg/L}] \ge 0.90.$$

Application Summary

The application of the technique and model to Higgins Lake resulted in a "most likely" phosphorus concentration of 0.0068 mg/L (Step 3), with 55% confidence limits bounding the "true" phosphorus concentration between 0.0039 mg/L and 0.0106 mg/L.

Relating back to the trophic classification in Table 2, Higgins Lake is probably:

1) oligotrophic
2) clear, and suitable for water-based recreation and a cold-water fishery.

These predicted trophic conditions in fact describe the present observed conditions in Higgins Lake. A median phosphorus concentration value of 0.006 mg/L was determined for Higgins Lake by the Environmental Protection Agency's National Eutrophication Survey (U.S.E.P.A. 1975).

Conclusions

The simplicity of this technique necessarily limits its adaptability to certain situations that may occur within a watershed. This procedure may be flexible enough to accommodate some of these situations, but others may require more intensive study (than the procedure provides for). Therefore, it must be left to the judgment of the user as to whether or not this method is appropriate. Examples of events or characteristics that would alter the effectiveness of this procedure are

1) the input of phosphorus from sources not considered in the method presented. These sources might include a large number of resident waterfowl in and around the lake or fertilizers applied to shoreline lawns;
2) the trapping of phosphorus by mechanisms not considered. These phosphorus traps might include aquatic plants or an upstream lake within the watershed;
3) the occurrence of an unnatural phenomenon that alters the lake ecosystem. These phenomena might include dredging, filling, and chemical treatment;
4) lake types not modeled well with this black box nutrient model. These types include closed lakes (lakes without well-defined outlets) and lakes with strong internal concentration gradients (i.e. lakes with significant local quality variations).

The technique presented herein needs to be considered within the broad context of lake management. It is important not to rely too heavily on lake models and projected lake trophic states for the determination of policy, since policy is not simply a technical issue. This technique merely provides information on the impact of watershed characteristics and activities on phosphorus concentration, which is a measure of lake trophic quality. This information is just one input for the analysis of lake management strategies. We need to know, in addition, the costs and benefits associated with suggested management options for the lake of study. Then, through the consideration of the effects of alternative strategies on all concerned groups, we may begin to manage our lakes wisely.

Acknowledgments

The authors would like to thank Mark Dawson for his assistance during the early stages of this paper, Howard Wandell and Richard Mikula for their review of a draft, and Steven Chapra for many helpful comments on the final draft. Portions of this work were supported by a grant from the National Oceanic and Atmospheric Administration, Contract No. 03-78-B01-109.

BENJAMIN, J. R., AND C. A. CORNELL. 1970. Probability statistics, and decision for civil engineers. McGraw-Hill, New York.

CHAPRA, S. C., AND K. H. RECKHOW. 1979. Expressing the phosphorus loading concept in probabilistic terms. J. Fish., Res. Board Can. 36: 225–229.

DILLON, P. J., AND F. H. RIGLER. 1975. A simple method for predicting the capacity of a lake for development based on lake trophic status. J. Fish Res. Board Can. 32: 1519–1531.

ELLIS, B. G., AND K. E. CHILDS. 1973. Nutrient movement from septic tanks and lawn fertilization. Michigan Dept. of Natural Resources, Tech. Bul. 73–5, Lansing, MI.

KING, D. L. 1979. Lake measurements. *In* Lake restoration: Proceedings of a National Conference. United States Environmental Protection Agency, EPA-440/5-79-001.

PROGRESSIVE ENGINEERING CONSULTANTS. 1976. Facilities plan for Lyon, Markey and Garrish Townships. Grand Rapids, MI.

RECKHOW, K. H. 1979a. Quantitative techniques for the assessment of lake quality. United States Environmental Protection Agency, EPA-440/5-79-015.

1979b. Sampling design for lake phosphorus budgets. Proc. of the American Water Resources Association Symposium on the Establishment of Water Quality Monitoring Programs, San Francisco, CA.

RECKHOW, K. H., M. N. BEAULAC, AND J. T. SIMPSON. 1980. Modeling phosphorus loading in lake response under uncertainty: a manual and compilation of export coefficients. U.S. Environ. Prot. Agency EPA-440/5-80-011.

RECKHOW, K. H., AND S. C. CHAPRA. 1979. The need for simple approaches for the estimation of lake model prediction uncertainty. International Institute for Applied Systems Analysis Task Force Meeting on "Uncertainty and Forecasting of Water Quality." Laxenburg, Austria.

RODIEK, R. K. 1979. Some watershed analysis tools for lake management. *In* Lake restoration: Proc. of a National Conference. United States Environmental Protection Agency, EPA-440/5-79-001.

TILSTRA, J. R., K. W. MALUEG, AND W. C. LARSON. 1972. Removal of phosphorus and nitrogen from wastewater effluent by induced soil percolation. J. Water Pollut. Cont. Fed. 44(5): 796–805.

TOFFLEMIRE, T. J., AND M. CHEN. 1977. Phosphate removal by sands and soils. Ground Water 15(5): 377–387.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (U.S.E.P.A.). 1975. National eutrophication survey working paper on Higgins Lake. U.S. E.P.A. Laboratory, Corvallis, OR.

UTTOMARK, P. D., J. D. CHAPIN, AND K. M. GREEN. 1974. Estimating nutrient loading of lakes from nonpoint sources. U.S. Environ. Prot. Agency EPA-660/13-74-020.

VALLENTYNE, J. R. 1974. The algal bowl. Fish. Mar. Serv. Misc. Spec. Publ. 22: 186 p.

VOLLENWEIDER, R. A. 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors. OECD Tech. Rep., Paris, DAS/CSI/68.21.

NOTES

Geosmin and 2-Methylisoborneol Implicated as a Cause of Muddy Odor and Flavor in Commercial Fish from Cedar Lake, Manitoba

M. YURKOWSKI AND JO-ANNE L. TABACHEK

Department of Fisheries and Oceans, Western Region, Freshwater Institute, Winnipeg, Man. R3T 2N6

YURKOWSKI, M., AND J.-A. L. TABACHEK. 1980. Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar Lake, Manitoba. Can. J. Fish. Aquat. Sci. 37: 1449–1450.

A muddy flavor and odor in commercial fish from Cedar Lake resulted in the closure of the commercial fishing season. Gas–liquid chromatography indicated the presence of geosmin and 2-methylisoborneol which are known to cause a muddy flavor in fish. The source of these compounds was not established but they are known to be produced by blue-green algae and actinomycetes.

Key words: organoleptic properties, geosmin, 2-methylisoborneol, commercial species, freshwater, Cedar Lake, Manitoba; *Stizostedion vitreum vitreum*, *Esox lucius*, *Coregonus artedii*, *Coregonus clupeaformis*

YURKOWSKI, M., AND J.-A. L. TABACHEK. 1980. Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar Lake, Manitoba. Can. J. Fish. Aquat. Sci. 37: 1449–1450.

A cause d'un goût et d'une odeur de vase des poissons commerciaux du lac des Cèdres, la pêche commerciale a été interdite. La chromatographie des gaz révèle la présence de géosmine et de 2-méthylisobornéol, substances reconnues comme produisant un goût de vase chez les poissons. Nous n'avons pas identifié la source de ces composés, mais on sait qu'ils sont produits par des algues bleu-vert et des actinomycètes.

Received November 21, 1979
Accepted May 21, 1980

Reçu le 21 novembre 1979
Accepté le 21 mai 1980

ON August 15, 1977, a complaint was received that a moldy or earthy odor and flavor was present in commercial walleye (*Stizostedion vitreum vitreum*) from Cedar Lake, Manitoba. When the next shipment of fish from Cedar Lake was inspected at the Freshwater Fish Marketing Corporation in Winnipeg, the same odors were noticed in walleye, northern pike (*Esox lucius*), cisco or lake herring (*Coregonus artedii*), and lake whitefish (*Coregonus clupeaformis*). Because of their unpalatable condition, the fish were considered unmarketable for human consumption and the commercial fishing season was closed on August 25, 1977 (E. W. J. Stubbington personal communication). The presence of a muddy–earthy flavor of slight to moderate intensity was confirmed by a taste panel of trained judges at the Freshwater Institute, Winnipeg (R. K. York and D. G. Iredale personal communication).

The presence of the compounds responsible for the flavor was objectively detected by a procedure previously reported (Yurkowski and Tabachek 1974). Minced walleye with slight to moderate muddy flavor was added to boiled distilled water and the mixture was distilled. The distillate was extracted with methylene chloride and analyzed by a gas–liquid chromatograph equipped with an effluent splitter. In all samples tested, two peaks were observed having odors resembling 2-methylisoborneol (retention time of 10.1 min at 61°C) and geosmin (retention time of 15.3 min at 95°C). In addition, two authentic compounds, 2-methylisoborneol synthesized by the method of Medsker et al. (1969) and geosmin, isolated from *Lyngbya aestuarii* (Tabachek and Yurkowski 1976), had the same odors and retention times as the respective peaks from the fish samples. This shows that both compounds were present and contributed to the muddy flavor in fish from Cedar Lake.

It has been reported that geosmin was present and was the cause of muddy flavor in rainbow trout (*Salmo gairdneri*) from natural sources (Yurkowski and Tabachek 1974). Persson and York (1978) found that 2-methylisoborneol was taken up by rainbow trout in continuous-flow aquaria and produced a muddy flavor in the muscle. However, this is the first report show-

Printed in Canada (J5872)
Imprimé au Canada (J5872)

ing the presence of 2-methylisoborneol in fish from natural sources. Both geosmin (Yurkowski and Tabachek 1974) and 2-methylisoborneol (Yurkowski unpublished data) also occurred in muddy-flavored sockeye salmon or kokanee (*Oncorhynchus nerka*) from Nesland Lake, Mt. Nebo, Saskatchewan (Yurkowski and Tabachek 1974).

The source of muddy flavor compounds in Cedar Lake was not determined. However, geosmin and 2-methylisoborneol have been reported to be produced by actinomycetes (Gerber 1969; Rosen et al. 1970) and/or blue-green algae (Tabachek and Yurkowski 1976).

After completion of the Grand Rapids hydroelectric power dam in 1964, the water level of Cedar Lake was raised from 831 ft (253 m) to a maximum of 842 ft (257 m) above sea level, resulting in the flooding of 600 mi^2 (155 400 ha) of shoreline (Moshenko and McGregor 1978) and covering of the existing vegetation. Cedar Lake is shallow (estimated mean depth 11.38 ft (3.5 m); maximum depth 40 ft (12.2 m) (A. J. Derksen, Manitoba Department of Mines, Resources, and Environment, personal communication)) and eutrophic. Any additional organic matter due to impoundment would enhance the growth of blue-green algae (Round 1957) such as the muddy-odor-producing organisms which were isolated from shallow eutrophic pothole lakes (Tabachek and Yurkowski 1976). Thus, water impoundment may have resulted in the presence of muddy flavor in fish which disrupted the commercial fishing season in Cedar Lake. This is an example of how the changing of a water system can induce biological changes which can have a deleterious effect on the commercial freshwater fishery.

Acknowledgments — The authors thank Ms R. K. York and Mr G. W. G. McGregor of the Freshwater Institute, and Mr A. J. Derksen of the Manitoba Department of Mines, Natural Resources and Environment, for the information and assistance they provided.

GERBER, N. N. 1969. A volatile metabolite of actinomycetes, 2-methylisoborneol. J. Antibiot. (Tokyo) 22: 508–509.

MEDSKER, L. L., D. JENKINS, J. F. THOMAS, AND C. KOCH. 1969. Odorous compounds in natural waters. 2-exo-hydroxy-2-methylbornane, the major odorous compound produced by several actinomycetes. Environ. Sci. Technol. 3: 476–477.

MOSHENKO, R. W., AND G. W. G. MCGREGOR. 1978. Data on the experimental commercial fishery at Cedar Lake, Manitoba, 1973. Manitoba Fisheries Management Branch, MS. Rep. No. 78-62: 63 p.

PERSSON, P.-E., AND R. K. YORK. 1978. Notes on muddy odour. II. Uptake of 2-methylisoborneol by rainbow trout (*Salmo gairdneri*) in continuous flow aquaria. Aqua Fenn. 8: 89–90.

ROSEN, A. A., C. I. MASHNI, AND R. S. SAFFERMAN. 1970. Recent developments in the chemistry of odor in water: the cause of earthy/muddy odor. Water Treat. Exam. 19: 106–119.

ROUND, F. E. 1957. Studies on bottom-living algae in some lakes of the English Lake District. Part III. The distribution on the sediments of algal groups other than Bacillariophyceae. J. Ecol. 45: 649–664.

TABACHEK, J. L., AND M. YURKOWSKI. 1976. Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba. J. Fish. Res. Board Can. 33: 25–35.

YURKOWSKI, M., AND J. L. TABACHEK. 1974. Identification, analysis, and removal of geosmin from muddy-flavored trout. J. Fish. Res. Board Can. 31: 1851–1858.

Brain Acetylcholinesterase Inhibition and Hepatic Activation of Acephate and Fenitrothion in Rainbow Trout (*Salmo gairdneri*)

J. F. KLAVERKAMP AND B. R. HOBDEN

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

KLAVERKAMP, J. F., AND B. R. HOBDEN. 1980. Brain acetylcholinesterase inhibition and hepatic activation of acephate and fenitrothion in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1450–1453.

Two factors, potency of brain acetylcholinesterase (AChE) inhibition and biotransformation by liver homogenate, were investigated to understand the temperature-dependent toxicity of fenitrothion (FTN) in rainbow trout (*Salmo gairdneri*) and the 600- to 1000-fold differences in concentration between FTN and another organophosphate (OP) insecticide, acephate (ATE), required to produce death in these trout. Concentrations required to produce 50% inhibition of brain AChE were similar for ATE and FTN, ~125 mmol/L and 80 mmol/L, respectively, whereas fenitrooxon (FTO), the oxidative desulfuration metabolite of FTN, was approximately five orders of magnitude more potent. Incubation with liver homogenate, however, demon-

Printed in Canada (J5884)
Imprimé au Canada (J5884)

strated that a more potent brain AChE inhibitor was produced from ATE, but not from FTN. It is concluded that hepatic biotransformation of FTN to FTO does not explain previous observations of FTN temperature dependency and differences in concentrations producing lethality.

Key words: acephate, acetylcholinesterase, biotransformation, brain, fenitrothion, fenitrooxon, liver

KLAVERKAMP, J. F., AND B. R. HOBDEN. 1980. Brain acetylcholinesterase inhibition and hepatic activation of acephate and fenitrothion in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 1450–1453.

Le pouvoir d'inhibition de l'acétylcholinestérase (AChE) du cerveau et la biotransformation par un homogénat de foie sont les deux facteurs étudiés dans le but de comprendre la toxicité dépendante de la température du fenitrothion (FTN) chez des truites arc-en-ciel (*Salmo gairdneri*) et les différences de concentration, allant de 600 à 1000 fois, entre FTN et un autre insecticide organophosphoré (OP), l'acéphate (ATE), requise pour provoquer la mort de ces truites. Les concentrations requises pour une inhibition de 50% de AChE du cerveau sont semblables pour ATE et FTN, ~125 mmol/L et 80 mmol/L respectivement, alors que le fenitrooxon (FTO), un métabolite de désulfuration oxydative de FTN, est plus puissant d'environ cinq ordres de grandeur. L'incubation avec homogénat de foie démontre cependant qu'un inhibiteur d'AChE du cerveau plus puissant est produit à partir de ATE, mais non de FTN. Nous concluons que la biotransformation hépatique de FTN en FTO ne suffit pas à expliquer des observations antérieures sur la dépendance de FTN de la température et les différences de concentration requise pour provoquer la mort.

Received November 30, 1979
Accepted May 22, 1980

Reçu le 30 novembre 1979
Accepté le 22 mai 1980

ORGANOPHOSPHATE (OP) insecticides having a phosphorate (P = 0) group react directly to inhibit acetylcholinesterase (AChE) and do not require biotransformation to produce toxic effects in vertebrates (Eto 1974). The phosphorothioate (P = S) OP insecticides have little, if any, direct inhibitory activity on AChE and are thought to be biotransformed in the liver to the more toxic phosphoroate analogs by oxidative desulfuration (Loomis 1974; Silver 1974).

A recent investigation (Duangsawasdi and Klaverkamp 1979) on the effects of temperature on the acute lethality of the phosphoroate acephate (*O,S*-dimethyl acetylphosphoramidothioate) (ATE) and the phosphorothioate, fenitrothion, (*O,O*-dimethyl-*O*-(3-methyl-4-nitrophenyl)-phosphorothioate) (FTN) to rainbow trout (*Salmo gairdneri*) demonstrated that FTN toxicity was temperature-dependent, whereas mortality due to ATE was affected very little, if at all, by temperature. Calculating Q_{10} values (Shifrer et al. 1974; Hoar 1975) to express the increase in relative toxicity with increases in temperature gave an average value of 1.3 for ATE-exposed fish and 3.3 for FTN-exposed fish. These values indicated that biochemical reactions, such as those involved in oxidative desulfuration, were involved in the temperature-dependent toxicity of FTN, whereas only physical processes were involved in ATE toxicity.

The objectives of this study were to investigate whether rainbow trout liver can biotransform in vitro these OP insecticides to more active brain AChE-inhibiting metabolites and to determine the AChE-inhibiting properties of fenitrooxon (FTO), the oxidative desulfuration metabolite of FTN. If evidence was obtained which demonstrated that the liver could activate FTN and that FTO was more potent in inhibiting AChE, it would support the hypothesis that FTN biotransformation is the biochemical process responsible for the temperature dependence of FTN toxicity. These studies may also provide insight into the 600- to 1000-fold difference in LC50 values between ATE and FTN (Duangsawasdi and Klaverkamp 1979).

Materials and methods — Adult rainbow trout of either sex, averaging about 29 cm in length and 400 g in weight, were used in this study. They were held for at least 3 wk prior to use in dechlorinated Winnipeg city tap water at 15°C and at a 12-h light:12-h dark photoperiod in 200-L tanks having a water supply flowing at 6 L/min.

Brain AChE inhibition studies on ATE, FTN, and FTO were conducted at 15°C using the manometric procedures previously described (Hobden and Klaverkamp 1977). "Premium" grade FTN and FTO of 99.0% purity (Sumitomo Chemical Co.) and "standard analytical" grade ATE of 99.31% purity (Chevron Chemical International) were incubated with brain homogenate at 15°C with constant shaking for 40 min prior to AChE activity determinations. OP concentrations are expressed as final concentrations in the incubation flask. Propylene glycol (50 μL/manometric flask), which has very little effect on this AChE activity (Hobden and Klaverkamp 1977), was used to solubilize FTN.

Fresh or boiled (100°C for 5 min) liver homogenate (50 mg/mL) was prepared using modified Cortland saline solution (Hobden and Klaverkamp 1977) fortified with 10^{-3} mol/L NADH and 10^{-4} mol/L NADPH (Gibson 1971).

TABLE 1. In vitro activation of ATE and FTN as brain AChE inhibitors by incubation in liver. Data are expressed as mean ± SD; $n = 4$ for each group. $^*P < 0.05$, $^{**}P < 0.01$.

Organophosphate	Percent inhibition of brain AChE before incubation in liver homogenate	Percent inhibition of brain AChE following incubation in fresh liver homogenate	Percent inhibition of brain AChE following incubation in boiled liver homogenate
Acephate (ATE) (3×10^{-3} mol)	6.3±2.6	14.7±1.6**	9.5±2.0*
Fenitrothion (FTN) (1×10^{-2} mol)	34.8±4.4	42.9±5.4*	39.2±7.0

ATE (99.31% purity) or FTN (99% purity) was incubated in this homogenate for 4 h at 15°C with constant shaking. An aliquot of this mixture, providing ATE at 3×10^{-3} mol/L and FTN at 10^{-2} mol/L was incubated with brain homogenate for 40 min at 15°C under identical conditions to those used to determine the brain AChE inhibition produced by ATE, FTN, and FTO. Inhibition of brain AChE activity was determined at 15°C using manometric procedures previously described (Hobden and Klaverkamp 1977).

In Table 1 comparisons for statistical significance of differences between percent inhibition of brain AChE before incubation in liver homogenate and after incubation in either fresh liver homogenate or boiled liver homogenate were made using paired *t*-tests.

Results — Concentration–effect curves for brain AChE inhibition produced by ATE and FTN are presented in Fig. 1. From these curves pI50 values (negative log of the concentration producing 50% inhibition) of 1.1 for FTN and, when the curve is extrapolated to 50% inhibition, of ~0.9 for ATE are obtained. A similar curve for FTO is presented in Fig. 1. The pI50 values of 5.8 obtained from this curve indicate FTO is almost five orders of magnitude more potent than ATE and FTN.

Incubation of ATE and FTN in fresh liver homogenate produced a greater increase of AChE inhibition than incubation in boiled homogenate (Table 1). The smaller, yet significant, increase in AChE inhibition produced by ATE in boiled homogenate is presumably due to "activation" by nonenzymatic processes, whereas the difference between AChE inhibition produced by ATE or FTN in fresh and in boiled liver homogenate represents activation by enzymatic processes. These differences were significant at the 0.01 probability level for ATE, but were not significantly different for FTN.

Discussion — There are many pharmacological/toxicological factors, such as rate of uptake, lipid solubility, detoxification reactions, excretion mechanisms (see Duangsawasdi and Klaverkamp 1979 for a synopsis of these factors), which may be involved in the differences between ATE and FTN in temperature dependency and concentrations required to produce mortality in rainbow trout. This study has examined two factors, potency of brain AChE inhibition and biotransformation, which may explain these differences.

The 600- to 1000-fold differences in LC50 values, for example 850 mg/L and 1.4 mg/L for ATE and FTN, respectively, at 96 h and 15°C (Duangsawasdi and Klaverkamp 1979) apparently are not due to brain AChE inhibitory potency. An approximate 50-fold difference in concentration, 2.6×10^{-2} mol/L for ATE and 5×10^{-4} mol/L for FTN was required to produce 20% inhibition of AChE activity. There was very little difference between ATE and FTN, however, in the concentration required to produce 50% inhibition, the

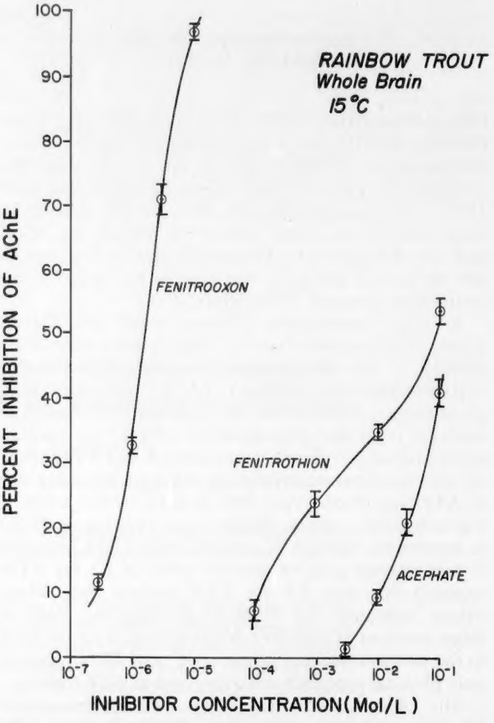


FIG. 1. Concentration–effect curves for brain AChE inhibition produced by ATE, FTN, and FTO. Data are expressed as the mean and the 95% confidence limits. $n = 6$ for each point.

pharmacological end point generally used for comparing potencies.

Since FTO has much greater AChE-inhibitory action, the biotransformation of FTN to FTO may explain the differences in concentrations required to produce mortality and the temperature dependency of FTN-induced mortality. In vitro incubation experiments have been used to demonstrate that parathion, another phosphorothioate, is activated to a more poten AChE-inhibiting compound, probably paraoxon, by liver homogenate preparations from brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (Potter and O'Brien 1964) and from bluegills (*Lepomis macrochirus*) and green sunfish (*Lepomis cyanellus*) (Gibson 1971). The liver homogenate experiments in this study, however, do not unequivocally demonstrate that in vitro liver preparations from rainbow trout can activate FTN by metabolic processes. While there was a slight, but significant, increase in brain AChE inhibition produced after incubation of FTN with fresh liver homogenate, this increase cannot solely be the result of enzymatic activation, since there was no difference between AChE inhibition produced by FTN after incubation with fresh or boiled liver homogenates.

Using radiolabeled FTN and FTO with purified liver microsomal preparations from rainbow trout, Miyamoto et al. (1979) have recently provided evidence that these fish can metabolize FTN to FTO at 24°C. Using these techniques, they also demonstrated glutathione-dependent *O*-demethylation of FTO, NADPH, and calcium-dependent hydrolysis of FTO to 3-methyl-4-nitrophenol, and glucuronide formation. These FTO deactivation reactions could have produced a decrease in FTO in this study, similiar to the in vitro detoxification reactions observed for methylparaoxon in the pumpkinseed (*Lepomis gibbosus*) (Benke et al. 1974).

When subjected to the same incubation procedure as FTN, an increase in AChE inhibition was observed with ATE in fresh and boiled liver homogenate. These were unexpected observations and may be due to deacetylation of ATE. Quistad et al. (1970) found that minor substitutions of groups on the nitrogen atom of *O,S*-dimethyl phosphoramidothioates, such as ATE, have great effect on the toxicity of these compounds and, in general, the simpler the substitution, the greater the toxicity of the compound. A deacetylated ATE compound, *O,S*-dimethyl phosphoramidothioate, has been produced as an insecticide for the control of lepidopterous larvae (Fest and Schmidt 1973).

Since the liver incubation experiments in this study demonstrate that ATE, but not FTN, was activated by metabolic processes, the enzymatic biotransformation of FTN to FTO does not appear to explain the differences in acute lethality and temperature dependency between ATE and FTN. Rate of uptake dissimilarities between the water-soluble ATE and the lipid-soluble FTN may simply be the reason for these differences. With a pK_a of 8.3, ATE would be ~95% ionized at pH 7.0 and consequently would not cross gill membranes as readily as the unionized FTN (Hunn and Allen 1974). Biochemical and physiological evidence, however, do not support this speculation, in that immediate and similar changes in serum and erythrocyte cholinesterase and in parameters, such as decreased heart rate and increased respiration rate, demonstrate that toxicological concentrations of ATE and FTN are taken up by rainbow trout in 1 h or less (Duangsawasdi and Klaverkamp 1979). Further studies on rates of uptake, accumulation, and physiological target sites of action in fish are required to understand the mechanisms involved in acute lethality and temperature dependence of these OP insecticides.

Acknowledgments — We thank Drs J. W. Clayton and W. L. Lockhart for their critical reviews.

BENKE, G. M., K. L. CHEEVER, F. E. MIRER, AND S. D. MURPHY. 1974. Comparative toxicity, anticholinesterase action and metabolism of methylparathion and parathion in sunfish and mice. Toxicol. Appl. Pharmacol. 28: 97–109.

DUANGSAWASDI, M., AND J. F. KLAVERKAMP. 1979. Acephate and fenitrothion toxicity in rainbow trout: effects of temperature stress and investigations on the sites of action, p. 35–51. *In* L. L. Marking and R. A. Kimerli [ed.] Aquatic toxicology. ASTM STP 667.

ETO, M. 1974. Organophosphorus pesticides: organic and biological chemistry. CRS Press, Cleveland, OH. 387 p.

FEST, C., AND K. J. SCHMIDT. 1973. The chemistry of organophosphorus pesticides. Springer-Verlag, NY. 339 p.

GIBSON, J. R. 1971. Comparative biochemistry of parathion metabolism in three species of fishes. Ph.D. thesis, Mississippi State University, State College, Mississippi. 22 p.

HOAR, W. S. 1975. General and comparative physiology. 2nd ed. Prentice Hall, Englewood Cliffs, NJ. p. 329–344.

HOBDEN, B. R., AND J. F. KLAVERKAMP. 1977. A pharmacological characterization of acetylcholinesterase from rainbow trout (*Salmo gairdneri*) brain. Comp. Biochem. Physiol. 576: 131–133.

HUNN, J. B., AND J. L. ALLEN. 1974. Movement of drugs across the gills of fishes. Annu. Rev. Pharmacol. 14: 47–55.

LOOMIS, T. A. 1974. Essentials of toxicology. 2nd ed. Lea & Febiger, Philadelphia. 223 p.

MIYAMOTO, J., Y. TAKIMOTO, AND D. MIHARA. 1979. Metabolism of organophosphorus insecticides in aquatic organisms, with special emphasis on fenitrothion, p. 3–20. *In* M. A. Q. Khan, J. J. Lech, and J. J. Menn [ed.] Pesticide and xenobiotic metabolism in aquatic organisms. ACS Symp. Ser. 99. Am. Chem. Soc., Washington, D.C.

POTTER, J. L., AND R. D. O'BRIEN. 1964. Parathion activated by livers of aquatic and terrestrial vertebrates. Science (Wash., D.C.) 144: 55–56.

QUISTAD, G. B., T. R. FUKUTO, AND R. L. METCALFE. 1970. Insecticidal, anticholinesterase and hydrolytic properties of phosphoramidothioates. J. Agric. Food Chem. 18: 189–194.

SHIFRER, C. C., E. J. MIDDLEBROOKS, D. B. PORCELLA, AND W. F. SIGHER. 1974. Effects of temperature on the toxicity of oil refinery waste, sodium chlorate and treated sewage to fathead minnows. Utah Water Research Laboratory, Utah State University, Logan, UT. 79 p.

SILVER, A. 1974. The biology of cholinesterase. North Holland Publishing Co., Amsterdam. 596 p.

Diel Activity Pattern and Swimming Speeds of Northern Pike (*Esox lucius*) in Lac Ste. Anne, Alberta

JAMES S. DIANA[1]

Department of Zoology, University of Alberta, Edmonton, Alta. T6G 2E9

DIANA, J. S. 1980. Diel activity pattern and swimming speeds of northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta. Can. J. Fish. Aquat. Sci. 37: 1454–1458.

Movements of six northern pike (*Esox lucius*) were monitored for 5 to 51 days by ultrasonic transmitters implanted surgically. The pike were inactive during 80% of the eight hundred and eighty-nine 5-min intervals monitored during summer and winter. No regular diel changes in activity were noted, except that pike were inactive at night. Swimming velocities calculated from gross displacements were maximum, 42 cm/s (0.91 body lengths (BL)/s); average, 23.1 cm/s (0.45 BL/s).

Key words: movements, diel activity, seasonal activity, northern pike, *Esox lucius*; swimming speeds

DIANA, J. S. 1980. Diel activity pattern and swimming speeds of northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta. Can. J. Fish. Aquat. Sci. 37: 1454–1458.

Nous avons suivi les mouvements de six grands brochets (*Esox lucius*) sur une période de 5 à 51 jours au moyen d'émetteurs ultrasonores insérés chirurgicalement. Les poissons étaient inactifs dans 80% des 889 intervalles de 5 min auxquels les observations ont été faites, en été et en hiver. Aucun changement nycthéméral régulier d'activité n'a été observé, sauf l'inactivité des grands brochets la nuit. Les vitesses de nage calculées par déplacements bruts étaient : maximales, 42 cm/s (0,91 longueurs corporelles (LC)/s); moyennes, 23,1 cm/s (0,45 LC/s).

Received December 12, 1978
Accepted June 4, 1980

Reçu le 12 décembre 1978
Accepté le 4 juin 1980

TELEMETRY has been used primarily to determine long-range movements of fish. Some studies have focused on locomotor behavior such as swimming speed, turn frequency, and diel activity patterns (Poddubnyi et al. 1970; Malinin 1971; Young et al. 1972; Kelso 1976a, b; Sciarrotta and Nelson 1977), in which fish were tracked for only a few days. Since stress of capture and handling could affect swimming behavior for several days (Summerfelt 1972; Diana et al. 1977), more reliable results on diel and seasonal changes in behavior would result from tracking over large time periods.

Diel changes in northern pike (*Esox lucius*) activity (Malinin 1969, 1971; Poddubnyi et al. 1970) and swimming behavior have been examined, but the results are difficult to assess. Pike are generally considered to be sedentary, lurking predators (Ivanova 1969; Nursall 1973). My hypothesis is that pike do not spend much time or energy in swimming, so that energy used in activity is not a very important factor in the energy budget of pike. To test this hypothesis, I have attempted to determine the time spent swimming and swimming speeds of northern pike using biotelemetry.

Methods — Tracking was conducted from March to July 1978, in Lac Ste. Anne, Alberta. The lake is moderate size (57 km^2), shallow (average depth 4.8 m), eutrophic, and located ~74 km northwest of Edmonton, Alberta. The lake is generally isothermal and temperatures are fairly constant in summer (~16°C from mid-May to October) and winter (ice cover, ~2°C from November to May). Six fish were captured by angling and implanted surgically with transmitters (Smith-Root Electronics, model SR69A), after Crossman (1977). One fish died 38 d after release, while the remainder survived for the duration of telemetric contact.

The fish were routinely located by long-range triangulation (Diana et al. 1977). Close-range tracking was conducted when possible from two observation stations ~100 m apart and 100 m from the fish. These stations were anchored boats during summer or holes through the ice during winter. The position of the fish was monitored simultaneously from both stations, and compass bearings of the fish's direction were recorded every 5 min for at least 1 h. Stations were relocated if the fish's movement led to a poor geometric relationship for triangulation. Observation intervals were varied, so all major day and night periods were sampled adequately (Table 1). After several hours of observation the locations of tracked fish were pinpointed by triangulation from the stations, and the distances be-

---

[1] Present address: School of Natural Resources, The University of Michigan, Ann Arbor, MI 48109.

Printed in Canada (J5467)
Imprimé au Canada (J5467)

TABLE 1. The number and percent occurrence (in parentheses) of pike active and inactive periods during 5-min-observation intervals at various times of day, both summer and winter. Inactive = movement less than 5 m (minimum movement that could be accurately detected). Sunrise period (Sr) = 1/2 h before to 1 h after sunrise, day (Dy) = sunrise period to sunset period, sunset period (Ss) = 1 h before to 1/2 h after sunset, and night (Ni) = sunset period to sunrise period. Range test = 4×2 χ^2 test; if $P > 0.05$, the symbols are underlined at the same level.

Time interval	Observations	Active	Inactive
	Summer		
Sunrise	87	19 (22%)	68 (78%)
Day	422	71 (17%)	351 (83%)
Sunset	78	16 (21%)	62 (79%)
Night	64	1 (2%)	63 (98%)
Total	651	107 (16%)	544 (84%)
Range test		Sr Dy Ss Ni	
	Winter		
Sunrise	39	9 (23%)	30 (77%)
Day	176	34 (19%)	142 (81%)
Sunset	75	26 (35%)	49 (65%)
Night	44	1 (2%)	43 (98%)
Total	334	70 (21%)	264 (79%)
Range test		Sr Dy Ss Ni	

TABLE 2. Calculated swimming velocities for four pike tracked during 1978 in Lac Ste. Anne, Alberta. $\bar{x} \pm$ SD. The values are based on displacements of 45 m or more in 5 min. A = total velocity (cm/s), B = relative velocity (body length/s). The number of these displacements is also given.

Fish No.	No. of displacements		Maximum velocity	Mean velocity
1	7	A	29	18.9 ± 4.7
		B	0.49	0.32 ± 0.08
2	2	A	18	17.2
		B	0.37	0.36
4	13	A	38	25.5 ± 6.6
		B	0.75	0.50 ± 0.13
6	6	A	42	25.9 ± 9.1
		B	0.91	0.56 ± 0.20
Total	28	A	42	23.1 ± 2.6
		B	0.91	0.45 ± 0.05

tween successive locations were determined by tape measure (when less than 36 m) or by an optical range finder (Ranging Inc., Rochester, NY).

Fish movements were generally detected by changes in bearings from both stations, and minimum detectable displacements were 2 m (winter) to 5 m (summer). No specific tests were conducted to determine the accuracy of this method, but the accuracy obtained appeared sufficient for reliable measurements of net movement over 5-min intervals. It was not feasible to use shorter time intervals, although several were tried. The fish were not monitored continuously, but close-range tracking was conducted sporadically for the duration of telemetric contact.

During the summer of 1976, a 15×1.8 m monofilament gill net with 6.4-cm-stretched mesh was set and lifted every 4 h over a 24-h period each month. During the summers of 1977 and 1978, a 91×1.8 m multifilament gill net with 6.4-cm mesh was set for at least one 3-h period in the afternoon during the 1st wk of each month. Total catch of pike was recorded during each of these sets.

Results — The long-range movements of six northern pike were examined for periods of 5 to 51 d. The fish did not limit their movements to a restricted home range (Fig. 1), and were found in the same type of habitat as determined previously (Diana et al. 1977). Pike showed similar long-range movements whether transmitters were implanted orally or surgically. However, fish with surgically implanted transmitters did not move for up to 5 d after implantation.

Northern pike were generally inactive, and displacements of 5 m or more occurred during less than 20% of all the 5-min observation intervals (Table 1). Data from all fish were combined, and the time intervals for grouping were chosen arbitrarily. During all periods of the day pike were most commonly inactive. Activity was sporadic, and often the fish did not move for up to 24 h. Pike were almost completely inactive at night during both summer (open water) and winter (ice cover) seasons. During both seasons, the only significant changes in activity were decreases at night. There were no significant differences between the activity pattern of pike in summer or winter ($\chi^2 = 2.71$, $P = 0.10$).

If one assumes that peak displacements during 5-min observations occurred as bouts of continuous straight line swimming, the distances moved can be used to calculate routine swimming speeds (Table 2). Any displacements of less than 45 m in 5 min were assumed to be due to nonlinear or discontinued movements. Only four fish exhibited this type of movement and 28 such displacements were measured. Maximum swimming speeds varied from 17 to 42 cm/s, and the mean swimming velocity was 23.1 cm/s. Swimming velocities were higher in summer than in winter, both in absolute (cm/s, $T = 2.77$, $P < 0.02$) and relative (body length (BL)/s, $T = 3.61$, $P < 0.01$) terms.

Gillnet catch of pike was also used to assess diel and seasonal changes in activity (Fig. 2). Diel activity patterns thus determined were similar to those found by telemetry, with a significant reduction in activity at night (22:00–02:00). More pike were caught in May and June than in late summer as was also found by Casselman (1978). Total gillnet catches should be reasonable indicators of activity, since the set times and locations were similar for all nets, thus total catch was proportional to catch per unit effort.

Discussion — Pike have long been considered sedentary (Ivanova 1969; Malinin 1969; Nursall 1973) and this study gives even more convincing evidence regarding this inactivity. Diel activity patterns found in the present study conflict with some results

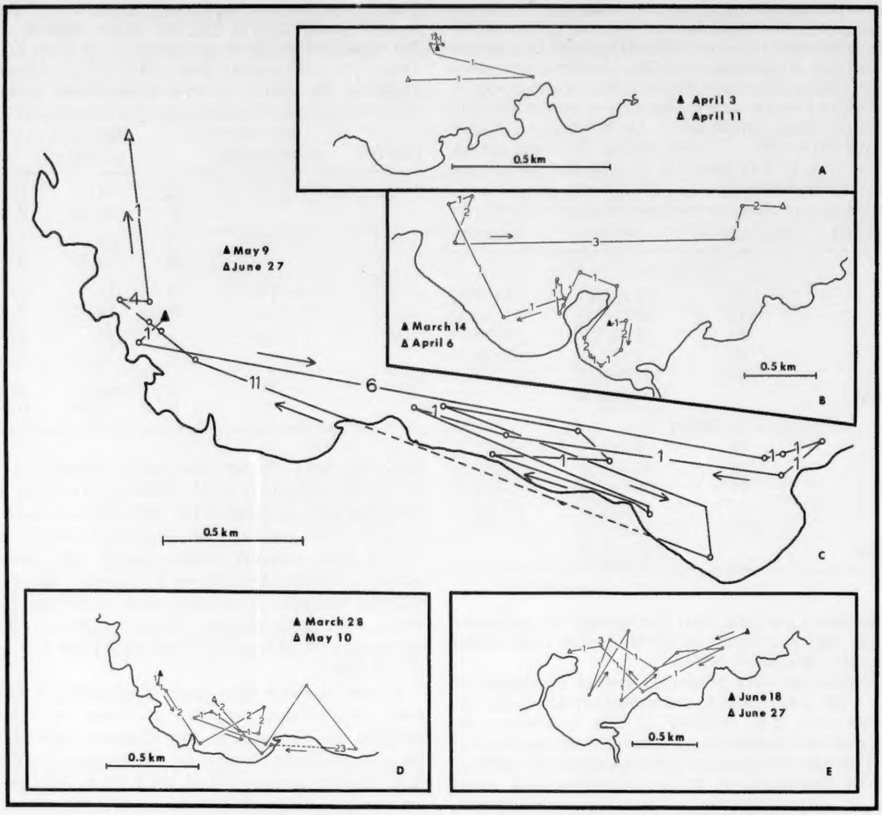


FIG. 1. Map of the locations of five northern pike tracked in 1978. Circles represent observed locations of the fish. Data from C and E are from summer tracks while A, B, and D are from winter observations. Closed triangles = release site; open triangles = last observed location. The number of days between observations are represented by numbers on the lines between circles.

of Lawler (1969), Malinin (1969), Poddubnyi et al. (1970), and Casselman (1978). However, both telemetry and gillnet data from Lac Ste. Anne indicate the same trend of diurnal activity and nocturnal inactivity. Peak activity during crepuscular or nocturnal periods from gillnet data (Lawler 1969; Casselman 1978) may be due to net avoidance during periods of high light intensity. Crepuscular activity patterns from direct telemetry observations (Malinin 1969, 1971; Poddubnyi et al. 1970) are more difficult to explain away, and it may be that pike in various locations have different activity patterns.

During 5-min-tracking intervals, pike activity could vary from nil to a constant swim. Swimming speed could vary as well, but for this analysis I assume that speed was constant. Calculations of swimming speed also assume that fish swam in a straight line. During the winter, the turn frequency (indicated by changes in the signal strength) during active movements averaged two per 5-minute period, indicating that pike rarely turned.

Jones et al. (1974) determined in the laboratory that the critical velocity (maximum sustainable velocity for 10 min) for pike of similar size to those I examined would be 44 cm/s (0.86 BL/s), and the highest maintained speed (for 100 min) was ~60% of the critical velocity, or 26 cm/s (0.52 BL/s). Both values are close to the maximum and mean speeds I observed for free swimming fish.

Poddubnyi et al. (1970) measured pike swimming velocities in a manner similar to mine, and found the mean speed to be 5.5 cm/s, with a maximum of 300 cm/s (over 1 min). The low mean values obtained indicate that they probably included inactive periods in

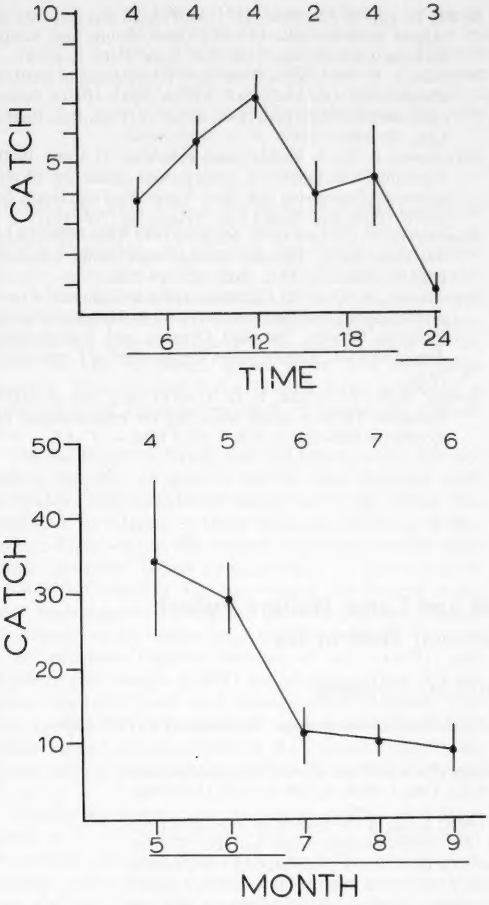


FIG. 2. Catches of pike in Lac Ste. Anne ($\bar{x}$ ± SE). Numbers across top = sets for each period. Time periods underlined at the same level had no significant differences in catch (Student–Newman–Keuls test, $P > 0.05$): (a) In a 15 × 1.8 m gillnet set and lifted every 4 h over a 24-h period each summer month in 1976. The time periods indicated are the midpoints of 4-h net sets, the only time period with complete darkness would be at 24:00 (22:00–02:00). (b) In a 91 × 1.8 m gillnet set for at least a 3-h period during each summer month in 1977 and 1978.

calculation of mean speed. The high value for maximum speed was probably due to a burst movement. It is doubtful that such a speed could be maintained over a 5-min interval (Jones et al. 1974), and I detected no speeds of this magnitude.

Calculated pike swimming velocities were significantly slower in winter than summer. Winter conditions (no vegetation, ice cover, cold temperatures) may influence swimming speed. Jones et al. (1974) found that pike swam slower at low temperatures in the laboratory, and Beamish (1978) indicated that this was true for most fish species.

A lack of evidence for localized home ranges here and in a previous study (Diana et al. 1977) indicate that pike showed similar movements whether transmitters were implanted orally or surgically. Since neither type of stress resulted in appreciably different behavior, the observed locomotor patterns may also be similar to those of undisturbed fish.

My observations of pike activity were measured during limited periods of the year. Gillnet results (Fig. 2) indicate that my summer measurements were taken during periods of maximum activity and may be overestimates. Since my conclusion is that pike are relatively inactive anyway, the timing problem probably did not affect the outcome of this study. Another problem may be the lack of resolution in tracking, since movements less than 5 m could not be detected. However, these slight movements should require very little time or very slow movements, which are probably unimportant in overall time–energy budgets.

Because of the low level of activity in northern pike from Lac Ste. Anne, I conclude that during most of the year activity is not an important part of a pike's energy budget. This allows one to equate actual metabolic rates in nature to routine metabolic rates in the laboratory. However, there are indications that pike may be much more active during the spawning period (Miller 1948; Moen and Henegar 1971; Casselman 1978), and activity may indeed be important at that time of year.

Acknowledgments — This study was supported by a Graduate Research Assistantship from the University of Alberta, and in part by NRCC grant No. A6587 to W. C. Mackay. The research was conducted out of the Lac Ste. Anne Fish Research Laboratory, University of Alberta. B. Boog, D. Goulet, and B. Medford aided in the tracking. I thank W. Mackay and B. Medford, who reviewed the manuscript. Special thanks are extended to Barbara Diana for her help and encouragement througout this study.

BEAMISH, F. W. H. 1978. Swimming capacity, p. 101–187. *In* W. S. Hoar and D. J. Randall [ed.]. Fish physiology, Vol. VII. Academic Press, New York.

CASSELMAN, J. M. 1978. Effects of environmental factors on growth, survival, activity, and exploitation of northern pike. Am. Fish. Soc. Spec. Publ. 11: 114–128.

CROSSMAN, E. J. 1977. Displacement and home range movements of muskellunge determined by ultrasonic tracking Environ. Biol. Fish 1: 145–158.

DIANA, J. S., W. C. MACKAY, AND M. EHRMAN. 1977. Movements and habitat preference of northern pike (*Esox lucius* L.) in Lac Ste. Anne, Alberta. Trans. Am. Fish. Soc. 106: 560–565.

IVANOVA, M. N. 1969. The behavior of predatory fish during feeding. J. Ichthyol. 9: 574–577.

JONES, D. R., J. W. KICENIUK, AND O. S. BAMFORD. 1974. Evaluation of the swimming performance of several fish species from the MacKenzie River. J. Fish. Res. Board Can. 31: 1641–1647.

KELSO, J. R. M. 1976a. Movement of yellow perch (*Perca flavescens*) and white sucker (*Catostomus commersoni*) in a nearshore Great Lakes habitat subject to a thermal discharge. J. Fish. Res. Board Can. 33: 42–53.

1976b. Diel movement of walleye, *Stizostedion vitreum vitreum*, in West Blue Lake, Manitoba, as determined by ultrasonic tracking. J. Fish. Res. Board Can. 33: 2070–2072.

LAWLER, G. H. 1969. Activity periods of some fishes in Heming Lake, Canada. J. Fish. Res. Board Can. 26: 3266–3267.

MALININ, L. K. 1969. Home range and homing instinct of fish. Zool. Zh. 48: 381–391. (Fish. Res. Board Can. Transl. Ser. No. 2050).

1971. Home range and actual paths of fish in the river pool of the Rybinsk reservoir. Biol. Vnutr. Vod Inf. Byull. 22: 158–166. (Fish. Res. Board Can. Transl. Ser. No. 2282).

MILLER, R. B. 1948. A note on the movement of pike, *Esox lucius*. Copeia 1948: 62.

MOEN, T., AND D. HENEGAR. 1971. Movement and recovery of tagged northern pike in Lake Oahe, South and North Dakota, 1964–68. Am. Fish. Soc. Spec. Publ. 8: 85–93.

NURSALL, J. R. 1973. Some behavioral interactions of spottail shiners (*Notropis hudsonius*), yellow perch (*Perca flavescens*), and northern pike (*Esox lucius*). J. Fish. Res. Board Can. 30: 1161–1178.

PODDUBNYI, A. G., L. K. MALININ, AND V. V. GAIDUK. 1970. Experiment in telemetric observations under ice of the behavior of wintering fish. Biol. Vnutr. Vod Inf. Byull. 6: 65–70. (Fish. Res. Board Can. Transl. Ser. No. 1817)

SCIARROTTA, T. C., AND D. R. NELSON. 1977. Diel behavior of the blue shark, *Prionace glauca*, near Santa Catalina Island, California. Fish. Bull. 75: 519–528.

SUMMERFELT, R. C. 1972. Flathead catfish movements. Completion report, Oklahoma project 4-60-R, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, United States Department of Commerce. 76 p.

YOUNG, A. H., P. TYTLER, F. G. T. HOLLIDAY, AND A. MACFARLANE. 1972. A small sonic tag for measurement of locomotor behavior in fish. J. Fish Biol. 4: 57–65.

Storage Characteristics of Small and Large Walleye Pollock (*Theragra chalcogramma*) Held in Ice

D. E. KRAMER[1] AND D. M. A. NORDIN

Department of Fisheries and Oceans, Vancouver Technological Research Laboratory, Vancouver, B.C. V6T 1X2

KRAMER, D. E., AND D. M. A. NORDIN. 1980. Storage characteristics of small and large walleye pollock (*Theragra chalcogramma*) held in ice. Can. J. Fish. Aquat. Sci. 37: 1458–1462.

Organoleptic changes and trimethylamine (TMA) levels in the muscle of small and large walleye pollock were determined after 0, 5, and 10 d of iced storage. Small pollock ($\leq$50 cm in length) spoiled appreciably faster than large pollock ($\geq$60 cm) as measured by organoleptic assessment of flavor and odor. Mean TMA levels in small and large pollock were not significantly different at any of the three iced storage times; however, variation among fish was large for both sizes after 10 d of iced storage. Also, TMA level along the length of individual pollock varied greatly, indicating whole fillets should be used for TMA analysis in this species. The size and kind of homogenizer used did not affect the efficiency of TMA extraction from the flesh.

Key words: walleye pollock, *Theragra chalcogramma*; iced storage, organoleptic assessment, trimethylamine

KRAMER, D. E., AND D. M. A. NORDIN. 1980. Storage characteristics of small and large walleye pollock (*Theragra chalcogramma*) held in ice. Can. J. Fish. Aquat. Sci. 37: 1458–1462.

Les changements organoleptiques et les niveaux de triméthylamine (TMA) dans le muscle de petites et grandes morues charbonnières ont été déterminés après 0, 5 et 10 jours d'entreposage dans la glace. Les petites morues ($\leq$50 cm de long) se gâtent beaucoup plus rapidement que les grandes ($\geq$60 cm), comme l'indique une évaluation organoleptique de la saveur et de l'odeur. Les niveaux moyens de TMA chez les petites et les grandes morues charbonnières ne

---

[1] Present address: University of Alaska, Marine Advisory Program, 2651 Providence Avenue, Anchorage, AK 99504.

Printed in Canada (J5671)
Imprimé au Canada (J5671)

diffèrent pas de façon significative à l'une ou l'autre des trois périodes d'entreposage; cependant, chez les deux groupes de taille, il y a forte variation après 10 jours d'entreposage dans la glace. En outre, la répartition, dans le sens de la longueur d'un poisson individuel, du niveau de TMA varie considérablement. Ce qui veut dire que des filets entiers devraient être utilisés dans l'analyse de la TMA chez cette espèce. La grandeur et le genre d'homogénéisateur utilisé n'affectent pas l'efficacité de l'extraction de la TMA des chairs.

Received June 14, 1979 Reçu le 14 juin 1979
Accepted June 5, 1980 Accepté le 5 juin 1980

During the past 3 yr, a walleye (or Alaska) pollock (*Theragra chalcogramma*) fishery has developed in British Columbia and landings have increased tremendously compared to the early 1970s. If a high demand for white fish flesh continues to develop, there will be a strong market for fillets from this species. Also, pollock roe can be recovered at certain times of the year for sale to Japan, where there is a very large market. Tsuyuki and Fuke (1978) have published a description of Japanese methods of processing pollock roe.

As the fishery expands and the larger, older fish are taken, the size of pollock landed may decrease considerably. This has already happened in the Bering Sea and Gulf of Alaska. In these areas, as well as in Washington State waters, the pollock are much smaller than those presently fished commercially in inside waters of British Columbia. For this reason, the present study was undertaken to compare the iced storage characteristics of small versus large walleye pollock.

In previous reports, Kramer et al. (1977) and Kramer and Nordin (1979) found that walleye pollock have relatively good iced storage characteristics. The fish sampled were medium to large in size, typical of those landed commercially in B.C. Overall quality of these samples changed very little with up to 8 d storage in ice.

Studies by Nelson and Miyauchi (1976) and Reppond et al. (1978) indicate that walleye pollock from the Bering Sea and Gulf of Alaska have much poorer storage characteristics when held in ice compared to our findings for pollock caught in inside B.C. waters. Dyer (1973) also notes that some reports from Japan show walleye pollock to have extremely poor refrigerated storage characteristics compared to cod.

The purpose of this study was to determine whether the iced storage characteristics, specifically organoleptic qualities and trimethylamine (TMA) changes, were significantly different for small and large walleye pollock. The variation in TMA along the length of the fish as well as influence of size and kind of homogenizer on the extraction of TMA were also checked.

Materials and methods — Walleye pollock used for this work were caught in October 1978 in Queen Charlotte Sound at a depth of 190 fathoms (1 fathom = 1.8288 m) using a bottom trawl. The fish were not actively feeding when caught as the stomachs contained very little food. Pollock 50 cm and under in total length were classified as "small" (mean weight 0.91 kg) while those 60 cm and over were considered "large" (mean weight 1.81 kg).

The first part of the study involved organoleptic assessment and TMA analysis of fillets from small and large pollock held as ungutted fish for 0, 5, and 10 d in ice. Eight small and four large pollock were gutted and frozen as controls. Sixteen small and 8 large pollock were placed in iced storage using an ice:fish ratio of 4:1. After 5 d storage in ice, eight small and four large pollock were removed, gutted, and frozen. After 10 d storage in ice, the remaining eight small and four large pollock were removed, gutted, and frozen.

After a minimum of 5 d and a maximum of 20 d frozen storage (from the first to last day of analysis), samples were thawed for 3 h in double (one inside the other) polyethylene bags in a 20°C water bath. Fillets were then removed and skinned. Left-side fillets were used for organoleptic assessment and right-side fillets were analyzed for TMA.

The left fillets were cut into six 1½ in. × 2 in. × 1 in. pieces (one for each taste panel member). These were individually wrapped in 5-in. squares of aluminum foil, steamed for 10 min, then scored by six trained taste panel members (according to the scoresheet given by Kramer et al. 1977). A mean score was calculated for each fish. These means were used to calculate a mean score for each group (small and large) at each iced storage time.

Analysis for TMA was carried out using the whole right fillet (white muscle only). The method used was that of Murray and Gibson (1972a, b) as described by Kramer et al. (1977). It involves extraction of the TMA with trichloroacetic acid followed by formation of a TMA–picrate complex.

The second part of this study was concerned with variation in TMA along the length of the fish. It involved holding five small (45–49 cm) walleye pollock in ice for 10 d, then gutting and freezing them prior to TMA analysis. Six 4-cm-thick steaks were cut between the tail and head region of each fish; this covered the region from which a fillet would be taken. These were analyzed for TMA (using all of the white muscle from each steak), following the method mentioned in the previous paragraph.

As use of whole fillets for the TMA determination (to eliminate possible variations along the length of the fish) required use of different size homogenizers, the final part of the study was concerned with the effect of homogenizer size on the amount of TMA extracted. Three different size homogenizers were compared:

1) Sorvall Omni-Mixer (medium size cup, total capacity 415 mL)
2) Waring Commercial Blendor, model 5011, Series B, 2 speeds (stainless steel container, total capacity 1075 mL)
3) Waring Blendor, model CB-2, 5 speeds (stainless steel container, total capacity 5615 mL).

Homogenizer number 1 was used in the second part of this work (determining if there are variations in TMA along

TABLE 1. Average organoleptic scores of small and large walleye pollock stored 0, 5, and 10 d in ice (SE are given in parentheses).

	Length		Average organoleptic score*				
Storage time in ice, d	Mean (cm)	Range (cm)	Flavor	Odor	Tenderness	Texture	Juiciness
0	Small 48	46–49	7.8 (0.2)	7.5 (0.1)	7.7 (0.1)[a]	7.1 (0.1)[b]	7.1 (0.1)
	Large 62	61–66	7.6 (0.2)	7.1 (0.2)	7.0 (0.2)[a]	6.4 (0.3)[b]	6.8 (0.2)
5	Small 46	41–49	6.2 (0.2)[c]	6.8 (0.1)[d]	7.7 (0.1)[e]	6.9 (0.1)	6.9 (0.1)
	Large 60	60–61	7.6 (0.2)[c]	7.4 (0.2)[d]	7.1 (0.2)[e]	6.6 (0.2)	6.9 (0.1)
10	Small 47	43–49	5.2 (0.2)[f]	5.5 (0.2)[g]	7.4 (0.2)	6.9 (0.1)	6.6 (0.1)
	Large 60	60–62	6.1 (0.2)[f]	6.5 (0.3)[g]	7.0 (0.2)	6.5 (0.1)	6.4 (0.1)

*For small fish, each value represents eight fish, each tasted by six panelists; for large fish, each value represents four fish, each tasted by six panelists.

[a,b,c,d,e,f,g] Means which have the same superscript are significantly different at $\alpha = 0.05$.

the length of the fish). Homogenizer number 2 was used for the small pollock and number 3 for the large pollock in the first part of the study.

Six small pollock were used for this comparison — two were gutted and frozen immediately after catching, two were iced for 5 d prior to gutting and freezing, and two were iced for 10 d prior to gutting and freezing. All samples were thawed in double (one inside the other) polyethylene bags for 3 h in a 20°C water bath, then filleted. One fillet from each fish was used for the TMA extractions (as per Murray and Gibson 1972a, b) using homogenizers 1 and 2, and the other was extracted with homogenizer 3.

Results and discussion — Small and large fish — There is a significant difference in rate of spoliage between small and large walleye pollock as measured by changes in organoleptic quality after 5 and 10 d of iced storage (Table 1). Initially, the smaller fish are more tender and have finer texture. There is little change in tenderness, texture, and juiciness during storage in ice for both small and large fish. Although size of fish does not influence changes in tenderness, texture, and juiciness, the small pollock have significantly lower flavor and odor scores than the large after 5 d of iced storage (as shown in Table 2, 8% of their flavor scores were considered undesirable). By 10 d, the small pollock have exceeded the limit of acceptability, with 29% of the flavor and odor scores undesirable compared to only 4% for the large samples at this iced storage time (see Table 2). The organoleptic scores reported in Table 1 for large walleye pollock after 10 d in ice are in agreement with the organoleptic data we reported previously for this species during iced storage (Kramer et al. 1977; Kramer and Nordin 1979). Nelson and Miyauchi (1976) indicate pollock from Alaskan waters can be held 3 d in ice with little change in quality but by the 6th d of iced storage, odor and texture changes render the flesh unfit for food. Although there were a few low flavor scores, our small pollock were acceptable organoleptically after 5 d of iced storage. According to Reppond et al. (1978), uncooked pollock from Alaskan waters had a fishy odor and soft flesh after 5 d of iced storage. The difference in size of our small pollock (averaging about 0.91 kg) compared to those being taken in Alaskan waters (averaging about 0.45 kg) probably accounts for the difference in storage characteristics as measured organoleptically for iced pollock from the two areas.

Figure 1 shows there is very little effect of fish size on mean TMA values for walleye pollock iced for 0, 5, and 10 d. However, there is an extremely large variation in TMA level among the samples (both small and large) iced for 10 d, as is shown by the lines which give the ranges. Small pollock varied the most at this time. Large pollock did not range as high in TMA as the small pollock (the high value for the large was less than half the high value for the small). The mean and range for the large pollock after 10 d of iced storage are similar to the values we reported previously (Kramer et al. 1977; Kramer and Nordin 1979). Reppond et al. (1978) also found similar levels of TMA in walleye pollock (from Alaskan waters) iced for 10 d using an ice:fish ratio of 5:1.

Although the means of the TMA values for small and large walleye pollock stored 10 d in ice indicate there is little difference between the two sizes with regard

TABLE 2. Percentage of undesirable scores (below 5) for flavor and odor of small and large walleye pollock stored 0, 5, and 10 d in ice.

			Undesirable scores (%)	
Storage time in ice, d	Size	Total no. scores	Flavor	Odor
0	Small	48	0	0
	Large	24	0	0
5	Small	48	8	0
	Large	24	0	0
10	Small	48	29	29
	Large	24	4	4

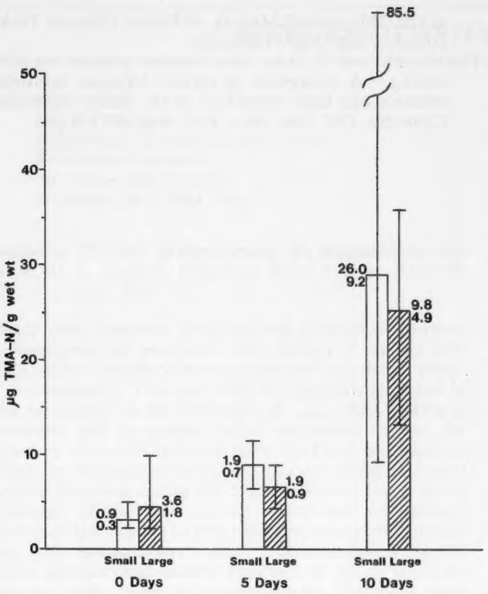


FIG. 1. Means and ranges of TMA levels in small and large walleye pollock stored 0, 5, and 10 d in ice. Upper numbers are standard deviations and lower numbers are standard errors.

to bacterial activity, the extremely large variation in the range of values found for the small fish shows that as a group, they will have a much greater range of quality loss after this period of iced storage. It is not clear whether this is due to small differences in handling and chilling or whether variation in some physiological condition between individual fish results in the smaller fish showing such a wide variation in TMA formation. The occurrence of very high levels of TMA in some of the small fish should result in those individuals receiving low flavor and odor scores and this agrees with the results in Table 2 where 29% (14 of 48) of the scores are considered undesirable.

The significantly different organoleptic scores for flavor and odor of large and small walleye pollock held 10 d in ice coupled with a difference in mean TMA values which is not significant should not be interpreted to mean TMA formation is a poor index of quality loss. For a proper correlation of these parameters, the TMA value for each fish should be compared with flavor and odor scores for that fish. This comparison has been made for both walleye pollock and Pacific cod in previous work (Kramer et al. 1977; Kramer and Nordin 1979), and we have insufficient data to improve on this.

Variation in TMA along the length of the fish — Three of the five pollock analyzed had highest TMA levels in the abdominal region. The other two had the highest level of TMA in the steak nearest the tail, with one of these having an extremely high TMA level in that steak compared to the rest of that fish. For all fish, the steaks lowest in TMA were from the head region. The ranges of TMA (μg/g wet wt) found in the five pollock were 79 to 191, 34 to 97, 12 to 122, 68 to 105, and 13 to 38. It appears that an accurate TMA determination in this species requires sampling by extraction of TMA from whole fillets rather than steaks.

Homogenizer effect — The size and kind of homogenizer used did not affect the efficiency of TMA extraction from the flesh. The small pollock used here had mean TMA values (μg/g wet wt) of 2.7 and 3.1 for the two frozen immediately after catching, 6.9 and 7.3 for the two fish iced 5 d, and 25.8 and 20.7 for the two fish iced 10 d. TMA values used to calculate the first mean value were 2.7, 2.9, and 2.6 and those for the last value were 21.9, 20.6, and 19.6 for homogenizers 1, 2, and 3, respectively (see Materials and methods). Thus the different homogenizers used in the first part of this work did not contribute to variations between the small and large pollock with regard to amount of TMA extracted.

Implications — The results of this work suggest that for all species it is very important to determine and report fish size in any studies on fish quality and fish storage characteristics. Fish size is too often omitted from the description of experimental procedures. If fillets are used and data on size of the round fish are not available, it would be wise to estimate from the fillet size the approximate original fish size. In many cases, a size difference may explain differences in storage properties found by workers in different laboratories for the same species.

Acknowledgments — We gratefully acknowledge the assistance of the following members of our staff who served as members of the taste panel: G. Gibbard, M. Ho, R. Hurst, M. Peters, and D. Smith.

DYER, W. J. 1973. New technical research on frozen fish. Refrigeration 48(543): 38–41.

KRAMER, D. E., AND D. M. A. NORDIN. 1979. Quality changes in Alaska pollock during frozen storage at −18°C. Fish. Mar. Serv. Tech. Rep. 852: 41 p.

KRAMER, D. E., D. M. A. NORDIN, AND L. J. GARDNER. 1977. A comparison of the quality changes of Alaska pollock and Pacific cod during frozen storage at −28°C. Fish. Mar. Serv. Tech. Rep. 753: 63 p.

MURRAY, C. K., AND D. M. GIBSON. 1972a. An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt — Part I. J. Food Technol. 7(1): 35–46.

1972b. An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt — Part II. J. Food Technol. 7(1): 47–51.

NELSON, R. W., AND D. MIYAUCHI. 1976. Preservation and quality characteristics of Alaska bottomfish and tests on minced pollock flesh. National Marine Fisheries Service, Pacific Utilization Research Center Processed Report S-761: 36 p.

REPPOND, K. D., F. A. BULLARD, AND J. COLLINS. 1978. Comparison of changes in walleye pollock held in ice and in CO_2-modified refrigerated sea water. Paper presented at the 29th Annual Meeting of Pacific Fisheries Technologists, San Diego, California.

TSUYUKI, H., AND S. FUKE. 1978. Alaskan pollock roe processing — A description of current Japanese industrial methods and their adaptation to the fishery in British Columbia. Fish. Mar. Serv. Tech. Rep. 851: 28 p.

BOOK REVIEWS/COMPTES RENDUS

Book review editor:
David P. Scott
Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

Descriptive Physical Oceanography. An Introduction. *3rd Edition. G. L. Pickard. Pergamon Press, Oxford, England. 1979. ix + 233 p. $9.95.*

Since 1964, Professor Pickard's text, *Descriptive Physical Oceanography*, has provided a wide variety of readers with a well-written introduction to the field of descriptive physical oceanography. This new 3rd (SI) edition was issued in order to convert to the SI system of units, thus making it compatible with its recently issued companion volume, *Introductory Dynamic Oceanography*, by Pond and Pickard. SI units are expected to come into exclusive usage in oceanographic literature during the 1980s following the eventual acceptance of the International Association of Physical Sciences of the Ocean (IAPSO) working group on symbols, units, and nomenclature's draft recommendation by the various international bodies. This text is not in strict accordance with those recommendations, retaining such well-beloved oceanographic units and symbols as ‰, σ_t, and even sverdrups whose use the working group wishes to discourage strongly. Such a compromise is undoubtedly necessary, especially with ‰ and σ_t which I suspect will persist in oceanographic usage far into this decade. To assist one in making the transformation from old to new units, this text contains a short appendix, giving the SI units of the common physical quantities and conversion factors between those units and the older mixed units.

But conversion of units was not the only revision done. While the organization, philosophy, and indeed much of the material is carried over from the earlier editions, descriptions of new and ongoing work and results have been slipped in here and there into the text. In most cases these changes have been inserted so carefully that only through section by section comparison with earlier editions can they be detected.

The basic structure of the book remains rooted in the classical descriptions following Sverdrup, Wust, and others. New and ongoing work in a region is discussed only after the classical framework has been established. These discussions of new work are usually quite brief and do not generally leave the reader with a definite picture of how the new results alter the classical picture. They rather only emphasize the incompleteness and weaknesses of the classical pictures and point out that even general descriptive models of basin scale circulation are not accepted without controversy.

It's unfortunate that no new figures were prepared for this edition. For example, during the discussion on the use of radionuclides as tracers, an appropriate section would be useful to both illustrate the technique and emphasize its agreement with the classical model. There are a number of other areas in which the discussion of new work, methods, or models would be greatly helped by the inclusion of a diagram.

This new 3rd edition will undoubtedly continue to remain the standard against which introductory texts will be measured throughout the new decade. While the text will not provide the reader with a description and understanding of the developing new ideas concerning the circulation and distribution of properties in the world ocean, it clearly provides an introduction to the basis and the methods from and by which the new ideas are developing. It will still be the book I will give to new employees and friends in answer to the question, what do physical oceanographers do and why?

R. ALLYN CLARKE

Atlantic Oceanographic Laboratory
Bedford Institute of Oceanography
Dartmouth, N.S. B2Y 4A2

(R-293)

Water Quality in Warmwater Fish Ponds. *Claude E. Boyd. Auburn University Agricultural Experimental Station, Auburn, Alabama. 1979. 359 p. $4.20.*

Water quality plays a more important role in intensive aquaculture than in probably any other field. Fish can exist at high stocking densities only in a suitable and well-balanced aquatic environment. Adequate water quality is thus crucial, but has been notoriously neglected in the aquaculture literature. There are thousands of papers dealing with water quality "problems" of pristine oligotrophic lakes, fewer on problematic eutrophic systems, and very few on productive fish ponds — limited usually to routine dissolved oxygen and nutrient monitoring. Whether the fish live or die, whether they grow and acquire acceptable standards and flavor for mass consumption, all depends on water quality in the pond. This paradox may perhaps be explained by the fact that fish farming has been an exclusive domain of "classic" fish biologists interested in maximum production while environmentally oriented researchers found better opportunities in other aquatic disciplines.

The book by Boyd, a well-published author, comes on the market at the right time to fill this gap. It provides everything that has been missing — a solid, unpretentious, and scientific approach to the environmental problems of intensive fish production and incorporating experimentation based primarily on the author's own long experience at one of the world's leading schools (Department of Fisheries and Allied Aquaculture, Auburn University). This book is not simply a review, as one might expect, but rather a firsthand account of the findings and achievements from this particular school over the past two decades, designed for the use of fisheries researchers and operators.

The book is divided into three major parts: principles of water quality, water quality management, and measurement of water quality. Part one covers fundamentals of physical limnology and hydrology, water chemistry (major ions as well as nutrient cycles), and aquatic plants (measurements and effects on fish). The chapter dealing with environmental requirements for fish production summarizes all the beneficial and adverse effects that water quality parameters (physical, chemical, and limnological) exert on fish stocks. The approach to presenting these factors is very modern: instead of static concentration ranges found in other literature, Boyd introduces the newest concepts of chemical equilibria, solubility, ionic speciation, and oxidation–reduction potentials. These chapters can be used to the advantage of any demanding researcher to fill the gaps in his chemistry background.

Part two covers practical corrective measures to improve or maintain suitable water quality in fish ponds: liming, fertilization by inorganic and organic fertilizers (showing calculation of dosages), etc. Fish feeding and its effect on water quality of both ponds and pond effluents are mention also, as well as the problems associated with diurnal or seasonal oxygen deficiencies and corrective measures to prevent fish kills by supplementary aeration. Phytoplankton control by chemical and biological means and other treatment methods to improve water quality in ponds are well covered.

Part three covers sampling methods for measuring of water quality, storage and preservation of water samples, and finally analytical procedures for chemical and phytoplankton analyses and primary productivity (oxygen method only). The brief review in Appendix I is very useful information, covering the fundamentals of analytical chemistry and including measurement techniques and calculation of results. This is very important for biologists with limited analytical background. It is a pity that the author omitted the calculation of ionic balance of the pond water and expression of the results of chemical analyses in milliequivalents or their percentage as used by water chemists. Although perhaps trivial, this calculation is surprisingly misunderstood by many.

The book is loaded with informative graphs, sketches, diagrams, and tables. One can see the author's educational background, and the skill in presenting experimental methods in a well-digested way is clearly evident. My only criticism of the book is that it is too Auburnian. Even a diagram showing a typical pattern of thermal stratification in a fish pond shows the hypolimnetic temperature of 20.5°C! Many basic management techniques (liming, fertilization, etc.) are presented (unintentionally) as Auburn inventions, disregarding some 3000 yr of Chinese and 800 yr of European tradition along this line. Although the author does not pretend to make general statements and sticks honestly to the warmwater fish pond type, he ignores all recent (and also not so recent) developments in other countries of the world. With a little more effort to include work from outside Auburn and a little more generalization, the book would have become an excellent source of reference for water quality problems in freshwater rather than warmwater fish ponds. Despite this, it is a very useful and comprehensive source of information for both aquatic scientists and practicing fish farmers in other climatic zones. They will find here almost everything they need. I highly recommend its purchase.

J. BARICA

Department of Fisheries and Oceans
Winnipeg, Man. R3T 2N6

(R-304)

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Prevalence of a trypanosome in Atlantic cod (*Gadus morhua*) especially in relation to stocks in the Newfoundland area **R. A. Khan, J. Murphy, and D. Taylor**

Spatial distribution of zooplankton and phytoplankton in British Columbian coastal waters **D. L. Mackas, G. C. Louttit, and M. J. Austin**

A computer technique for estimating the size of sexual maturity in crabs **D. A. Somerton**

Daily growth rings in the otoliths of juvenile sockeye salmon (*Oncorhynchus nerka*) **K. H. Wilson and P. A. Larkin**

Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects **M. N. Charlton**

Characteristics of filamentous bacteria isolated from a gill disease of salmonids **H. Wakabayashi and S. Egusa**

Bacteria associated with fin rot disease in hatchery-reared Atlantic salmon (*Salmo salar*) **R. Schneider and B. L. Nicholson**

Enhancement of the survival of rainbow trout (*Salmo gairdneri*) eggs and fry in an acid lake through incubation in limestone **J. M. Gunn and W. Keller**

The role of fishes in the regulation of phosphorus availability in lakes **B. S. Nakashima and W. C. Leggett**

Gravel bed composition in Oregon coastal streams **J. N. Adams and R. L. Beschta**

Slow swimming and cyclical "fishing" behavior in *Aglantha digitale* (Hydromedusae: Trachylina) **G. O. Mackie**

Thermal habitat partitioning by fishes in Lake Michigan **S. B. Brandt, J. J. Magnuson, and L. B. Crowder**

Diurnal variation in the feeding intensity and catchability of Silver Lake (*Merluccius bilinearis*) **R. E. Bowman and E. W. Bowman**

An examination of Cd, Cu, and Hg concentrations in livers of Northern pike, *Esox lucius*, and white sucker, *Catostomus commersoni*, from five lakes near a base metal smelter at Flin Flon, Manitoba **G. A. McFarlane and W. G. Franzin**

Notes

Subitaneous and resting eggs of copepods: relative rates of clutch production by *Diaptomus leptopus* **C. J. Watras**

ANNOUNCEMENTS / AVIS

International Symposium on Quality of Groundwater — ISQG '81

This international symposium will be held March 23–27, 1981 at Leeuwenhorst Congres Center, 3 Langelaan, 2211 XT Noordwijkerhout (35 km from Amsterdam), The Netherlands. The aim of the symposium is to stimulate information between researchers and legislative and executive authorities. It will deal primarily with problems concerning aquifers of unconsolidated sediments: effects of diffuse polluting sources, land use, and precipitation; effects of local polluting sources; subsurface waste disposal and deep-well injection; hazardous pollutants in subsoil; collection and interpretation of data; development of models (with emphasis on hydrological, physical–chemical, and biological aspects); criteria and methods for protection of groundwater quality.

The conference languages will be English, French, and German with facilities for simultaneous translation during the plenary meetings. The proceedings will be published in English. Preprints will be available during the symposium.

The symposium is sponsored by UNESCO, WHO, EC, IAH, and IAHS. It is being organized by the Netherlands National Institute for Water Supply.

For further information please contact the congress office:

ISQG '81 c/o Klvl, P.O. Box 30424, 2500 GK The Hague, The Netherlands. (Telephone: (0) 70-646800, Telex: 33641 Kivi nl, Telegrams: Koninsting, The Hague.)

Colloque international sur la qualité de l'eau souterraine — ISQG '81

Ce colloque international se tiendra du 23 au 27 mars 1981 au Centre des congrès Leeuwenhorst, 3 Langelaan, 2211 XT Noordwijkerhout (à 35 km de Amsterdam), Pays-Bas. Le but du colloque est de stimuler l'échange d'information entre les chercheurs et les autorités législatives et exécutives. On y traitera surtout de problèmes concernant les formations aquifères des sédiments non consolidés : effets des sources diffuses de pollution, de l'utilisation des terres et de la précipitation; effets des sources locales de pollution; rejet des eaux usées sous la surface et injection de puits profonds; polluants dangereux dans le sous-sol; collection et interprétation des données; élaboration de modèles (avec accent sur les aspects hydrologiques, physico–chimiques et biologiques); critères et méthodes de protection de la qualité de l'eau souterraine.

L'anglais, le français et l'allemand seront les langues utilisées pour les conférences, avec traduction simultanée lors des séances plénières. Les comptes rendus seront publiés en anglais. Des préimprimés seront disponibles durant le colloque.

Ce colloque est patronné par UNESCO, WHO, EC, IAH et IAHS. Il est organisé par le *Netherlands Institute for Water Supply.*

Pour plus amples renseignements, on est prié de s'adresser au bureau du colloque :

ISQG '81, Boîte postale 30424, 2500 GK La Haye, Pays-Bas. (Téléphone : (0) 70-646800, Telex : 33641 Kivi nl, Télégrammes : Koninsting, La Haye.)

BULLETIN 191

Computation and interpretation of biological statistics of fish populations*

W. E. RICKER

(382 p., Cat. no Fs 94-191; Canada $10.50, other countries $12.60)

Available from:

Supply and Services Canada, Canadian Government Publishing Center, Hull, Que., Canada K1A 0S9

Please make cheques and money orders payable to the Receiver General for Canada. Advance payment in Canadian funds is required.

---

*Available in Russian from:
Pischevaya Promyshlennost
12 Pervy Kadashevsky per
Moscow 113035, USSR

BULLETIN 191F

Calcul et interprétation des statistiques biologiques des populations de poissons*

W. E. RICKER

(410 p., N^o de cat. Fs 94-191; Canada $10.50, autres pays $12.60)

S'adresser à :

Approvisionnements et Services Canada, Centre d'édition du gouvernement du Canada, Hull (Qué.), Canada K1A 0S9

Les chèques ou mandats-postes, payables à l'avance en monnaie canadienne, doivent être faits a l'ordre du Receveur général du Canada.

**Version russe disponible à :*
Pischevaya Promyshlennost
12 Pervy Kadashevsky per
Moscov 113035, URSS

Canadian
Journal
of Fisheries
and Aquatic
Sciences

(Formerly the *Journal of the Fisheries Research Board of Canada*)

Journal
canadien
des sciences
halieutiques
et aquatiques

(Anciennement le *Journal de l'office des recherches sur les pêcheries du Canada*)

CJFSDX37(9) 1337–1466(1980)
ISSN 0706-652X

Volume 37, No. 9, September 1980

Volume 37, n° 9, septembre 1980

A new approach to length–frequency analysis: growth structure **J. Schnute and D. Fournier** 1337–1351
Herbivore diet affects fecal pellet settling **P. K. Bienfang** 1352–1357
Reciprocal averaging and polar ordination as techniques for analyzing lotic macroinvertebrate communities **J. M. Culp and R. W. Davies** 1358–1364
Ammonia and nitrate uptake in the Lower Great Lakes **T. P. Murphy** 1365–1372
Factors affecting Puget Sound coho salmon (*Oncorhynchus kisutch*) runs **S. B. Mathews and F. W. Olson** 1373–1378
Influence of parental smolt age and sea age on growth and smolting of hatchery-reared Atlantic salmon (*Salmo salar*) **J. K. Bailey, R. L. Saunders, and M. I. Buzeta** 1379–1386
Sedimentological and biological changes in the Windsor mudflat, an area of induced siltation **T. R. Turk, M. J. Risk, R. W. M. Hirtle, and R. K. Yeo** 1387–1397
Circulation and energetics of a deep, strongly stratified inlet **H. J. Freeland and D. M. Farmer** 1398–1410
Resource partitioning in two coexisting sunfish: pumpkinseed (*Lepomis gibbosus*) and northern longear sunfish (*Lepomis megalotis peltastes*) **D. R. Laughlin and E. E. Werner** 1411–1420
Models of ammonia excretion for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) **L. J. Paulson** 1421–1425
Some sources of variability in determination of digestive enzyme activity in zooplankton **P. Mayzaud** 1426–1432
Relationship between thermal stability and summer oxygen depletion in a prairie pothole lake **M. H. Papst, J. A. Mathias, and J. Barica** 1433–1438
A procedure using modeling and error analysis for the prediction of lake phosphorus concentration from land use information **K. H. Reckhow and J. T. Simpson** 1439–1448

Notes

Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar Lake, Manitoba **M. Yurkowski and J.-A. L. Tabachek** 1449–1450
Brain acetylcholinesterase inhibition and hepatic activation of acephate and fenitrothion in rainbow trout (*Salmo gairdneri*) **J. F. Klaverkamp and B. R. Hobden** 1450–1453
Diel activity pattern and swimming speeds of northern pike (*Esox lucius*) in Lac Ste. Anne, Alberta **J. S. Diana** 1454–1458
Storage characteristics of small and large walleye pollock (*Theragra chalcogramma*) held in ice **D. E. Kramer and D. M. A. Nordin** 1458–1462

Book reviews/Comptes rendus: Descriptive physical oceanography. An introduction (G. L. Pickard) **R. A. Clarke**; Water quality in warmwater fish ponds (C. E. Boyd) **J. Barica** 1463–1464

Coming next month/À paraître le mois prochain 1465
Announcements/Avis 1466

© Minister of Supply and Services Canada 1980
Ministre des Approvisionnements et Services Canada 1980